ARCHIVES OF PATHOLOGY

VOLUME 47 MARCH 1949 NUMBER 3

FORMATION OF HEMOSIDERIN AND HEMATOIDIN AFTER TRAUMATIC AND SPONTANEOUS CEREBRAL HEMORRHAGES

GEORGE STRASSMANN, M.D.
WALTHAM, MASS.

THE FORMATION of hemosiderin and hematoidin subsequent to injuries and hemorrhages of the brain was studied early by Virchow [1] and later on by Langhans,[2] Quincke,[3] Neumann [4] and Duerck.[5] Since that time the reactions following cerebral injuries and hemorrhages in men and animals have been described by a number of investigators [6]; detailed reports have been given recently by Rand and Courville,[7] Hicks,[8] Baggenstoss, Kernohan and Drapiewski [9] and Tedeschi.[10] Still opinion differs as to the exact time at which histiocytes containing hemosiderin and (or) hematoidin appear after traumatic and spontaneous cerebral hemorrhages. For this reason experiments were made with mice; the results were compared with autopsy observations of man.

MATERIAL AND METHODS

Under chloroform anesthesia, stabbing wounds of one or both hemispheres were made with a thin needle in 50 white mice. In 11 of these mice two or three punctures were made at different times. The mice were killed at intervals ranging

From the Laboratory of the Metropolitan State Hospital.

1. Virchow, R.: Virchows Arch. f. path. Anat. **1**:379, 1847.
2. Langhans, T.: Virchows Arch. f. path. Anat. **49**:66, 1870.
3. Quincke, H.: Virchows Arch. f. path. Anat. **95**:125, 1884; Deutsches Arch. f. klin. Med. **25**:567, 1880; **27**:193, 1880.
4. Neumann, S.: Virchows Arch. f. path. Anat. **111**:25, 1888; **177**:401, 1904.
5. Duerck, H.: Virchows Arch. f. path. Anat. **130**:29, 1892.
6. Tschistowitsch, T.: Beitr. z. path. Anat. u. z. allg. Path. **23**:321, 1898. Cone, W.: Arch. Neurol. & Psychiat. **20**:34, 1928. Macklin, C., and Macklin, M.: ibid. **3**:353, 1920. Penfield, W.: Surg., Gynec. & Obst. **39**:803, 1934. del Río Hortega, P., and Penfield, W.: Bull. Johns Hopkins Hosp. **41**:278, 1927. Penfield, W., and Buckley, R.: Arch. Neurol. & Psychiat. **20**:1, 1928. Russell, D.: Am. J. Path. **5**:451, 1929. Wilson, R.: Arch. Neurol. & Psychiat. **15**:75, 1926. Winkelman, N., and Eckel, J.: ibid. **31**:956, 1934. Hassin, G.: ibid. **36**:231, 1946.
7. Rand, C., and Courville, C.: Arch. Neurol. & Psychiat. **22**:738, 1931; **27**:605 and 1342, 1932; **31**:527, 1934; **36**:1277, 1936; **55**:79, 1946.
8. Hicks, S. P.: Arch. Path. **43**:15, 1947.
9. Baggenstoss, A.; Kernohan, J., and Drapiewski, J.: Am. J. Clin. Path. **13**:333, 1943.
10. Tedeschi, C.: Arch. Neurol. & Psychiat. **53**:333, 1945.

from three hours to thirty days after the injury. The head was severed from the neck immediately after death and put into 4 per cent formaldehyde solution for twenty-four to forty-eight hours. Then the brain was removed from the skull, fixed again for some time in formaldehyde solution, embedded, cut and stained. For the iron stain Gömöri's modification of Perls's reaction was used.[11]. The results were compared with the findings in 40 persons with traumatic and 30 with spontaneous cerebral hemorrhages and (or) thromboses associated with hemorrhages whose brains were examined for the presence of iron-containing and iron-free blood pigment.[12] The duration of the hemorrhage was known in most cases from the history or from the onset of the clinical symptoms. The time of survival varied from thirty-seven hours to several years. All age groups were present. Most of the patients with spontaneous cerebral hemorrhages and thromboses were over 60 years old.

RESULTS

Mice.—Nervous disturbances resulting from the stabbing wounds and lasting from nine hours to four days were observed in 3 mice. Interrupted compulsory spinning of the body in a circle and loss of equilibrium were seen. Hemorrhages were found in the cerebellum, the basal ganglions and the cornu ammonis at autopsy. As a rule, the mice, after a short period of unconsciousness or drowsiness, recovered fast and behaved later like normal mice. No infections of the wounds occurred. The tracks of the stabbing wounds were often discovered only after several microscopic sections had been stained for iron. The tracks were small. The traumatic reactions varied considerably in different animals. First, edema occurred around the hemorrhagic area, then proliferation and detachment of vascular cells. Polymorphonuclear leukocytes were rarely seen. The histiocytes originated more often from endothelial and adventitial cells of the blood vessels than from microglia cells migrating to the injured area. The effused red cells had disappeared and had generally been engulfed by histiocytes after four to five days. These histiocytes were of varying size and shape—round, elongated or polygonal. They were found outside of blood vessels or in the walls of blood vessels. There was not much difference between histiocytes filled with hemosiderin and situated in the walls of vessels four days or thirty days after the injury.

Hemosiderin became visible in a few instances after forty-eight hours and regularly after seventy-two hours. The cytoplasm of the histiocytes took a pale bluish stain with Perls's test. After the fourth day the number of histiocytes increased, and the hemosiderin became darker blue, filling the whole cell as a diffuse mass or as an accumulation of granules. In stains other than the specific one for iron it was often difficult to discover the wound track and the histiocytes with hemo-

11. Perls, M.: Virchows Arch. f. path. Anat. **39**:42, 1867. Gömöri, G.: Am. J. Path. **12**:655, 1936.

12. In most of the cases of traumatic hemorrhages the specimens were observed and collected at autopsies performed by the Office of the Chief Medical Examiner of New York, Dr. Thomas Gonzales, who gave me permission to use his material.

siderin. They filled the tracks at any time between the fifth and the thirtieth day. (The experiments were not extended over thirty days.) In several instances the proliferated blood vessels and the histiocytes had a starlike appearance radiating from the center to the periphery. Fine yellow granules of hematoidin were discovered in histiocytes in 1 mouse eleven days after the injury; in a second mouse histiocytes

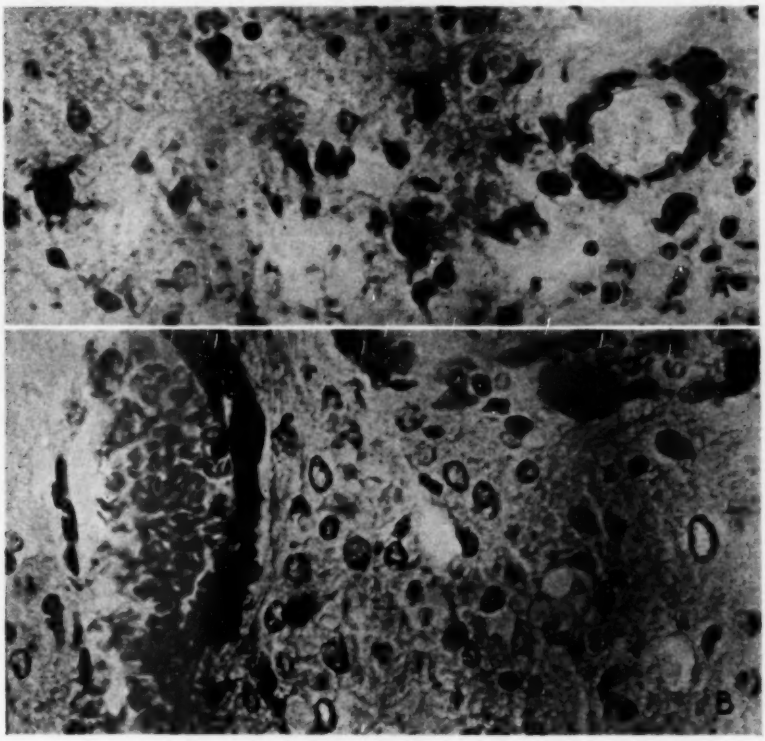


Fig. 1.—*A*, mouse wound track after four days, showing histiocytes filled with hemosiderin outside of blood vessels and in the walls of blood vessels. Hemosiderin stain, × 660.

B, mouse wound track after thirty days, showing similar histiocytes filled with hemosiderin around blood vessels and in the walls of vessels. Hemosiderin stain, × 660.

with hematoidin and histiocytes with hemosiderin were found in the wound track thirteen days after the injury; in all others the search for hematoidin revealed none. Around the wound track after seven days in

several cases there were areas of demyelination and vacuoles, proliferated astrocytes and astrocytic fibrils, and proliferated connective tissue fibers. The nerve cells and the glia cells of the areas adjacent to the wound track appeared normal. Large histiocytes without blood pigment, filled with lipoid material, were also seen.

Man.—In traumatic and spontaneous cerebral hemorrhages histiocytes containing hemosiderin appeared on the sixth day.[13] These histiocytes had originated from endothelial and adventitial vascular cells and from microglia cells. The number of the histiocytes with hemosiderin increased after this period, and the hemosiderin stained

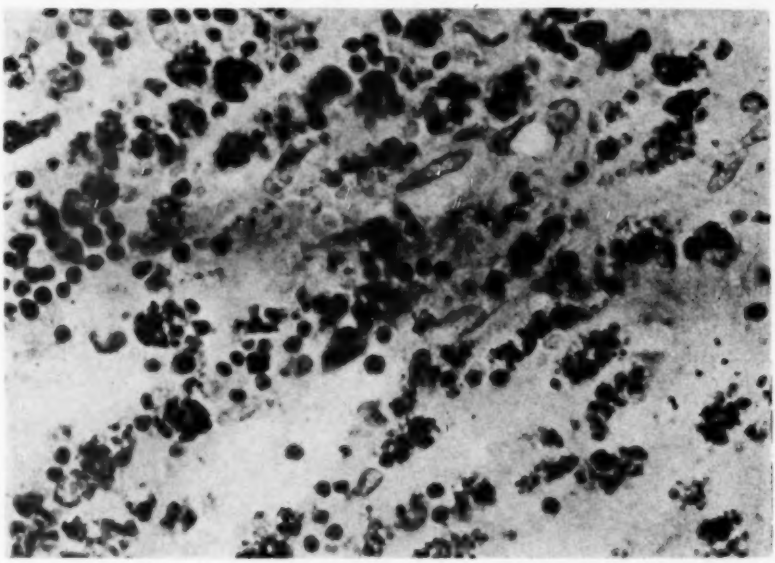


Fig. 2.—Border of a hemorrhagic area of the brain of a 40 year old man one month after the bleeding occurred. Numerous histiocytes with hematoidin, appearing black in the picture, and other histiocytes with a mixture of pigments or with hematoidin alone may be seen. Hemosiderin stain, × 660.

darker blue; some histiocytes invaded perivascular spaces and vascular walls early after the onset of the hemorrhage but most of them remained at the border of the hemorrhagic area for an indefinite period of months and years. Histiocytes containing yellow granules of hematoidin, not giving the iron reaction, became visible in and near the hemorrhagic area ten days after the onset of a spontaneous cerebral hemorrhage in 2 cases. More often hemosiderin was seen in one layer of cells at the

13. Strassmann, G.: Arch. Path. **38**:76, 1944; J. Neuropath. & Exper. Neurol. **4**:393, 1945.

outer border and hematoidin in an equal number of histiocytes at the inner border after fourteen days in traumatic and spontaneous cerebral hemorrhages. Hematoidin appeared later than hemosiderin. A mixture of both pigments was found in some cells, but these cells were less numerous than the histiocytes containing hemosiderin or those containing hematoidin. In a 40 year old man with hypertension two cerebral hemorrhages were observed. The one had occurred one month before death; the other one, ten days before death. The month-old hemorrhage showed an equal number of histiocytes with hemosiderin and (or) with hematoidin (fig. 2). The number of histiocytes with hematoidin was much smaller than the number of histiocytes with hemosiderin in the ten day old hemorrhage.

Generally, besides histiocytes with the blood pigments, numerous histiocytes filled with lipoid material were noted in older hemorrhagic areas. Histiocytes with hemosiderin and hematoidin and with lipoid stayed in these areas for an indefinite period. But in many cases of smaller cerebral hemorrhages or old subdural hemorrhages and in lobotomy scars of months' and years' duration, only histiocytes with hemosiderin and histiocytes with lipoid material were seen. In other cases of older softenings caused by vascular occlusion, no histiocytes with blood pigment and only histiocytes with fatty material were observed.

COMMENT

Rich [14] has proved that in tissue cultures mesodermal histiocytes split the hemoglobin from engulfed red cells into an iron-containing compound and the iron-free hematoidin. Muir and Niven,[15] twenty-four hours after injecting blood subcutaneously into rabbits, rats and mice, observed histiocytes containing hemosiderin; histiocytes with hematoidin appeared in rats and mice on the seventh day after the injection. No hematoidin was found in rabbits. In my own experiments [13] alveolar histiocytes containing hemosiderin became visible in rabbits thirty-three hours after intratracheal introduction of blood. In brain injuries of mice histiocytes with hemosiderin appeared occasionally after forty-eight hours and regularly after seventy-two hours. Histiocytes with hematoidin could be discovered in 1 mouse eleven days after the injury. In a second mouse histiocytes with hematoidin and histiocytes with hemosiderin were found in the wound track thirteen days after the injury. Hammes [16] observed histiocytes with iron pigment in subarachnoid hemorrhages of man on the third day. Baggenstoss, Kernohan and Drapiewski[9] saw such histiocytes between the fifth and the seventh day after ventricular punctures. In my own cases of traumatic and

14. Rich, A. R.: Bull. Johns Hopkins Hosp. **35**:415, 1924.
15. Muir, M., and Niven, J.: J. Path. & Bact. **41**:177 and 182, 1935.
16. Hammes, E.: Arch. Neurol. & Psychiat. **52**:505, 1944.

spontaneous cerebral hemorrhages hemosiderin became visible within histiocytes on the sixth day and hematoidin within histiocytes occasionally on the tenth day but more often after fourteen days. The arrangement of two layers of histiocytes, the outer one containing histiocytes with hemosiderin and the inner one histiocytes with hematoidin, near the hemorrhagic area was conspicuous. This fact was stressed as early as 1888 by Neumann.[4] In accordance with Virchow's opinion, it was believed for a long time that only hemosiderin is formed by the action of phagocytic cells and that hematoidin is formed in dead tissue independent of, and distant from, such cells. If hematoidin was observed within cells, it was thought that this iron-free pigment had been engulfed after its extracellular formation.[17] Already in 1884 Quincke doubted that this statement was correct. Since Rich's successful experiments the opinion now is generally accepted that hematoidin, also, is formed by the action of, and within, phagocytic cells. The presence of hematoidin could be demonstrated in histiocytes in our own material ten to fourteen days after the onset of the cerebral hemorrhage. Perhaps the small amount of effused red cells is responsible for the fact that in many instances no hematoidin but only hemosiderin was found. Some pigment may have been dissolved during the embedding process. Histiocytes with hemosiderin were always found earlier after blood was injected into animal tissue (the subcutaneous tissue,[16] the lungs [18] or regional lymph nodes [16]) than after cerebral hemorrhages in man. Apparently, histiocytes were in larger numbers and more easily and faster activated in the animal tissues and organs than in the brain of man. For the same reason histiocytes with blood pigment may appear earlier in subarachnoid hemorrhages than in injuries and hemorrhages of the brain itself, in which the healing process is slow. Two conclusions seem to be justified: (1) Histiocytes with hemosiderin appear on the fifth or sixth day after cerebral injuries and hemorrhages; (2) histiocytes with hemosiderin and histiocytes with hematoidin become visible after ten to fourteen days.

SUMMARY

The formation of hemosiderin and hematoidin was studied in injuries of the brains of mice and in traumatic and spontaneous cerebral hemorrhages of man. Hemosiderin appeared earlier after blood was injected into animal tissues or after injuries of the brains of mice than after cerebral hemorrhages in man. It seems probable that scarcity of available histiocytes and their slow activation explain the late formation of the human blood pigments.

17. Hueck, W.: Beitr. z. path. Anat. u. z. allg. Path. **54**:68, 1912. Lubarsch, O.: Klin. Wchnschr. **4**:2136, 1925. Schmidt, M. B.: Ergebn. d. allg. Path. **35**:105, 1940. Virchow.[1] Langhans.[2] Neumann.[4] Duerck.[5]

18. Moritz, A. R.: The Pathology of Trauma, Philadelphia, Lea & Febiger, 1942, p. 33.

SIGNIFICANCE OF EHRLICH'S REACTION IN CASES OF MELANURIA

A. J. LEA, M.D.
NORCROSS, BLACKPOOL, ENGLAND

EHRLICH'S test for indole and its substitution derivates have now been in use for many years, especially in bacteriologic work.[1] The test is simple and consists of the addition of an equal volume of Ehrlich's reagent to the solution suspected of containing indole bodies. The reaction is also given by solid indole derivatives. When indole or its derivatives is present, a "fine red colour"[2] develops in the cold. Ehrlich's reagent consists of para-dimethylaminobenzaldehyde, 95 per cent ethyl alcohol and concentrated hydrochloric acid in the proportions 4-380-80.[2] The red color is also given by pyrrole, skatole, glucosamine, urobilinogen and notably tryptophane. Apparently it is due to the pyrrole ring, though by no means all substituted or condensed pyrrole derivatives react positively. Rohde,[3] discussing the reaction with tryptophane in 1905, stated: *"Ueber die Natur der farbigen Verbindungen der Aldehyde mit der Skatolaminoessigsäure kann ich heute nur Vermutungen äussern"* (Concerning the nature of the colored combining of the aldehyde with the skatole aminoacetic acid I can at present express only a guess). The position has not changed appreciably in the ensuing forty-three years inasmuch as, apart from the probability that quinone formation is involved, nothing more is known of the composition of "rosindole," as the red substance is now called.

While it is recognized that normal urines frequently contain indole derivatives in quantities sufficient to give a positive reaction with Ehrlich's reagent, the test is nevertheless often used for the detection of melanuria and is regarded as the most sensitive of such tests.

From the Medical Services, Ministry of Pensions.

This work has been carried out with the aid of a grant from the Government Grant Committee of the Royal Society.

1. Fellers, C. R., and Clough, R. W.: J. Bact. **10**:105, 1925. Marshall, W. E.: J. Hyg. **7**:581, 1907.

2. Cole, S. W.: Practical Physiological Chemistry, ed. 9, Cambridge, Heffer and Sons, 1933.

3. Rohde, E.: Ztschr. f. physiol. Chem. **44**:161, 1905.

(According to Snell and Snell,[4] one part of indole per million can be detected by this method.) The purpose of this paper is to show that Ehrlich's reaction is a test not for melanin but only for melanogen and that a negative reaction can occur with true melanuria.

EXPERIMENTS

During the course of work on melanin it was desired to investigate the pigment of melanuria. A specimen of urine was obtained from a patient known to have Addison's disease. This gave a strongly positive reaction with Ehrlich's reagent but showed no evidence of melanin formation. The urine was acidified (it was originally faintly alkaline to litmus) and allowed to stand for two days, at the end of which time a deposit of melanin had formed. This deposit was filtered off and, purely fortuitously, the urine again was tested with Ehrlich's reagent. The result was negative. This was attributed to a complete filtering off of the melanin, though that was considered somewhat surprising as it is difficult to remove the whole of this pigment when it is in a state of comparative purity (as it is in melanuria) by simple filtration. Some of the removed melanin was replaced in the urine, but the result was still negative.

Investigation of the Tyrosine-Tyrosinase Reaction by Ehrlich's Test

Time	Result of Adding Ehrlich's Reagent
2115	Nil
2145	Nil
2215	Nil
2245	Nil
2315	Very faint pink color
2345	Deeper pink
0015	Still deeper pink
0045	Fainter pink
0115	Still fainter pink
0200	Nil
0300	Nil
0400	Nil
0600	Nil
0800	Nil

A fresh solution of para-dimethylaminobenzaldehyde was prepared in alcohol and hydrochloric acid and tested against a standard indole solution (1 cc. containing 0.02 mg. of indole). A strongly positive reaction occurred. Fresh addisonian urine was obtained, and again it gave a positive reaction. The urine was acidified and the melanin allowed to deposit for two days as before, but this time the pigment was not removed by filtration. Ehrlich's test was again carried out, and no reaction appeared. The test was then applied to known melanin from a melanoma, to that from the ink sac of Sepia officinalis and to the artificial pigment prepared by tyrosinase oxidation of tyrosine. The results, both with solid melanin and with solutions, were uniformly negative.

The oxidizing of tyrosine to melanin by tyrosinase was then followed by means of the Ehrlich test. Two cubic centimeters of a dilute mushroom extract was added to 100 cc. of a 0.02 per cent solution of tyrosine buffered to p_H 7.41 (electrometric). The experiment was carried out at room temperature without shaking. Five-tenths cubic centimeter of the mixture was removed periodically and an equal quantity of Ehrlich's reagent added to it. The results obtained are given in the table.

4. Snell, F. D., and Snell, C. T.: Colorimetric Methods of Analysis, New York, D. van Nostrand Co., Inc., 1937, vol. 2.

The reaction became negative again at about the time when a medium brown coloration had developed in the reacting mixture. To the unaided eye melanin formation appeared to be complete by 0400. Obviously a faintly positive reaction might have been masked by the melanin which had formed, although melanin added in varying amounts to a standard indole solution did not hide the color which developed on addition of Ehrlich's reagent. However, the experiment was repeated under the same conditions except that the reaction mixture was filtered before Ehrlich's reagent was added, and, to aid observation, the test was carried out on a white spotting tile. The result was the same. The experiment was again repeated, this time with a 1 per cent solution of tyramine hydrochloride, which is more readily soluble than tyrosine, to increase the probability of a faint positive reaction being observed. Once more the reaction, after becoming positive, became negative again shortly after melanin formation was evident.

There are two obvious explanations of these findings: (1) that melanin is not a polymerized indole derivative as is generally assumed, and (2) that polymerized indole derivatives do not give a positive reaction with Ehrlich's reagent. At present there does not appear to be any possibility of deciding which, if either, of these alternatives is correct, as no authentic indole polymers are known to which the Ehrlich test can be applied for comparison. Recent work by Mason [5] supports the view that melanin is an indole polymer.

The details of the oxidizing of tyrosine to melanin, as far as they are known, have been established by Raper [6] and are, briefly, as follows: tyrosine (para-hydroxyphenylalanine) takes up oxygen with the formation of 3,4-dihydroxyphenylalanine (dopa) which is then further oxidized to the orthoquinone. The next step is molecular rearrangement with formation of the indole ring. This indole derivative (5,6-dihydroxy-2,3-dihydroindole-2-carboxylic acid) is also converted into the corresponding orthoquinone, which in its turn passes through either 5,6-dihydroxyindole or 5,6-dihydroxyindole-2-carboxylic acid to melanin. The final stages of this reaction and the nature of the pigment itself are still unknown. It appears that the most probable explanation of the behavior of the Ehrlich test during this reaction is that it becomes positive with the formation of 5,6-dihydroxy-2,3-dihydroindole-2-carboxylic acid and that it continues to give positive reactions until the actual formation of the melanin, as all the aforementioned intermediate metabolites (with the exception of the second quinone, which is red) are colorless, and that it does not give a negative result again until melanin formation is definite to the unaided eye.

The immediate clinical importance of these findings is that the Ehrlich reagent is a test for melanogen in urine but not for melanin. It follows that a urine which shows a dark brown deposit and which does not give a positive reaction with Ehrlich's reagent may yet be

5. Mason, H. S.: J. Biol. Chem. **172**:83, 1948.

6. Raper, H. S.: Biochem. J. **20**:735, 1926.

that of a case of true melanuria in which the indole derivatives (melanogens) have all been oxidized to melanin. This may well happen with acid urines, especially with specimens sent through the post, i. e., where there is delay in examination.

SUMMARY

Evidence is produced to show that Ehrlich's reagent (para-dimethylaminobenzaldehyde) is not a test for melanin but is a test only for the propigment.

This work has been carried out with the aid of a grant from the Government Grant Committee of the Royal Society.

NONPARASITIC, NONCANCEROUS CYSTIC TUMORS OF THE SPLEEN

WARREN L. BOSTICK, M.D.
Assistant Professor of Pathology
AND
S. P. LUCIA, M.D.
Professor of Medicine
SAN FRANCISCO

ALTHOUGH cystic tumors of the spleen are rare, they are more common than the solid variety. Cystic tumors are less likely to be reported in the medical literature than solid tumors, possibly because the former are usually benign and often their cellular derivation is obvious. There are, however, some reports of cancerous cystic tumors of the spleen and some references to cystic tumors in which the cellular derivation is obscure.

A general survey of cystic tumors of the spleen was presented by Fowler,[1a] who classified them into two large groups: (1) the "true cysts," which included those tumors having a demonstrable cellular lining membrane, and (2) the "false" or "pseudocysts," which included all cysts devoid of a cellular lining layer. In the former group the cellular origin of the tumor is easily determined, while in the latter group no classification based on cellular origin is possible. Fowler [1b] recognized the weakness of the original term "pseudocyst" and subsequently adopted the term "secondary cyst."

The cells involved in the formation of most of the primary cystic splenic tumors are obvious. The group which needs the greatest clarification is the group of so-called epidermoid cystic neoplasms. In instances where elements of ectodermal origin are evident—e.g., hair, keratin and sebaceous glands—the classification is clearcut, and these tumors are properly called splenic dermoid tumors. A more difficult problem of classification occurs in those cases in which the cysts are lined by several layers of squamous cells which often possess intercellular bridges but are without keratinization or epidermal appendages. These tumors are generally known as "epidermoid" cysts and constitute about 10 per cent of all nonparasitic cystic tumors of the spleen. They are

From the Divisions of Pathology and Medicine, University of California Medical School.

1. Fowler, R. H.: (*a*) Ann. Surg. **57**:658, 1913; (*b*) Internat. Abstr. Surg. **70**:213, 1940.

probably more frequent than has been generally recognized. Custer [2] found 5 in 5,000 autopsies, and we have seen 2 in addition to the one reported in this paper.

Within a period of five years the authors have observed 3 cases of splenic tumors, in each of which there was presented a palpable upper abdominal mass and other interesting clinical and pathologic features. After complete clinical study, all 3 cases were diagnosed as probable instances of splenic tumors—cystic tumor being considered most likely in 2 of the cases.

REPORT OF CASES

CASE 1.—The patient's past history and the family history were irrelevant. The present illness began five years before entry. While the patient was exercising at school a slight pain developed in the lower part of the abdomen, which gradually over a period of several hours became quite severe. A physician was consulted, who discovered an enlarged spleen but did not make a definitive diagnosis. Subsequently there was noted residual mild discomfort, but only rarely did the patient experience severe pain. Occasionally he noticed pain in the left shoulder and some fulness in the upper part of the abdomen. There was no past history of jaundice, malaria or pertinent infectious diseases. He had always lived in the San Francisco metropolitan area.

The patient was a thin, underdeveloped young man, aged 20. The only abnormal findings were limited to the abdomen, where there was a prominent bulge and mass in the left upper quadrant, which moved with respiration. On palpation the mass was noted to be firm, nontender, round and smooth. The tumor extended from the ninth rib superiorly at the midclavicular line to the pelvic brim inferiorly, and laterally from the umbilicus to the flank. No other organs or masses were palpated.

Examination of the blood gave the following data: hemoglobin, 88 per cent (12.1 Gm.); red cells, 5,200,000; white cells, 5,400, with polymorphonuclear neutrophils 57, eosinophils 2, lymphocytes 26 and monocytes 15 per cent; platelets, 350,000; sedimentation rate (Wintrobe) 6 mm. in one hour; bleeding time (Duke), 2 minutes; clotting time (Lee and White), 3 minutes; fragility of red cells, 0.42 to 0.32; volume index, 0.949; color index, 0.791. Bone marrow obtained by puncture showed percentages as follows: myeloblasts, 0.6; promyelocytes, 11.2; neutrophilic myelocytes, 4.6; eosinophilic myelocytes, 7.6; nonfilamented polymorphonuclear neutrophilic leukocytes, 35; filamented polymorphonuclear neutrophilic leukocytes, 4.0; eosinophilic polymorphonuclear neutrophilic leukocytes, 1.6; lymphocytes, 11; monocytes, 5; plasma cells, 1; proerythroblasts, 2.4; erythroblasts, 16. Icterus indexes were 5 to 10. The Wassermann and Kahn tests were negative. The urine was normal. The bengal rose dye excretion test showed 60 per cent retention at 8 minutes and 42 per cent retention at 16 minutes. The intravenous hippuric acid test—one hour urine sample, 460 cc.—showed 0.86 Gm. of hippuric acid. The sputum was normal. No acid-fast bacteria were found.

The clinical diagnosis was: splenomegaly, probably cystic.

A left paracostal incision revealed an oval, enlarged, apparently cystic spleen which was practically free of adhesions and was easily removed. The liver and the gallbladder were grossly normal. The patient made an uneventful recovery.

---

2. Custer, R. P., in Brennemann, J.: Practice of Pediatrics, Hagerstown, Md., W. F. Prior Company, Inc., 1944, vol. 3, chap. 20.

Pathologic Report.—The specimen consisted of a spleen measuring 22 by 10 by 6 cm. and weighing 1,265 Gm. (fig. 1). It was grossly lobular because of the presence of six irregular cysts varying from 4 to 12 cm. in diameter. The surface was blue-gray except over the cysts, where the pale connective tissue was evident. When the largest cyst was opened, 580 cc. of thick, brown-colored fluid filled with cholesterol crystals was released. The lining of this cyst and of the smaller ones was smooth, glistening and characterized by trabeculation. Around the larger cysts there were small satellite cysts and focal areas of fibrosis.

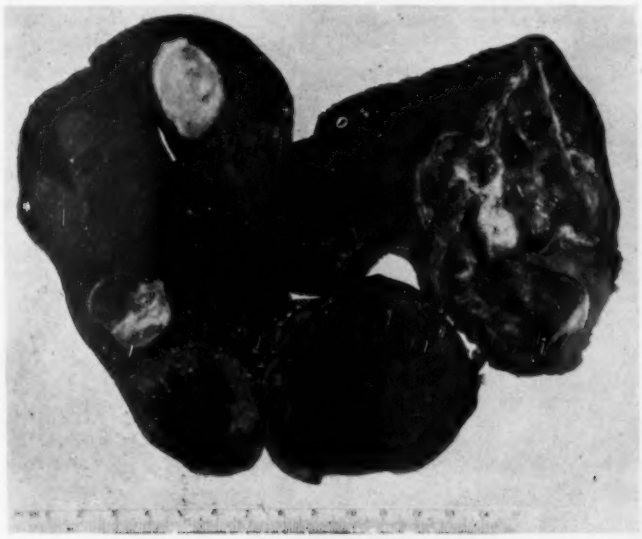


Fig. 1 (case 1).—Multiple separate cysts are evident in a spleen weighing 1,265 Gm. The largest one has been drained of its fluid, and the coarsely trabeculated wall is seen. In the smaller cysts the fluid has solidified after fixation.

The cysts were lined by squamous cells, many of which were swollen and cuboidal (fig. 2). Frequently, these cells were arranged in double or triple layers. At a few points, six or seven layers of squamous cells were noted. There was no keratinization, but some of the cells had distinct intercellular bridges. Hairs and glandular elements were absent. The cysts were either empty or filled with an amorphous pink-staining material containing a few macrophages.

Beneath the cyst wall there was a variable amount of connective tissue which merged gradually into the surrounding splenic tissue. In the splenic tissue the malpighian bodies were well formed, not hypertrophied, and contained normal central arterioles. The pulp was normal except for increased deposition of intersinusoidal connective tissue.

Diagnosis.—Multiple metaplastic mesodermal ("epidermoid") cysts of the spleen.

Case 2.—A 26 year old married white woman complained of a mass in the left upper quadrant of the abdomen, which was moderately tender. The family history was irrelevant. Five years before the present entry a diagnosis of "abdominal

cyst" was made, and on surgical intervention an enlarged spleen and three cysts were encountered. One cyst was located in the right ovary, and two were joined together above, and not attached to, the ovary. These cysts were removed, and the patient was told that they weighed 13 pounds (about 6 kilograms). No further detail concerning the cysts or the spleen was available. Following the operation she had been well except for some soreness of the left upper abdominal quadrant and evidences of a gradually enlarging mass in that region. One month before the present entry, while dancing, the patient first noted a rapidly enlarging lump in the base of the left side of the neck. It grew to 3 cm. in size and then remained

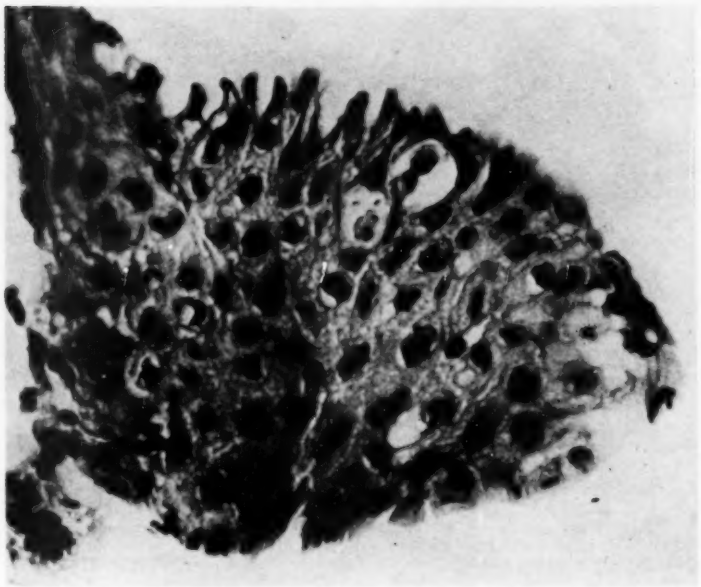


Fig. 2 (case 1).—High magnification of a portion of the multilayered squamous cell lining of one of the cysts. Intercellular bridges are present; keratinization is absent.

unaltered to the time of examination, a period of three weeks. In addition, the patient had a small red hemorrhagic tumor of the left index finger, which she stated became enlarged with each menstrual period and then regressed.

The pertinent findings were limited to the abdomen and the neck. The spleen was felt 13 cm. below the left costal margin and extended to the midline; its surface was coarsely nodular, and it was tender at the inferior pole. The liver was not enlarged. No other masses or abdominal abnormalities were noted. In the left cervical region, to the right of the insertion of the sternocleidomastoid muscle, was a soft, rubbery, easily movable mass measuring about 3 cm. in diameter.

The hemoglobin was 74 per cent (10.2 Gm.). The blood counts were: red cells, 4,610,000; white cells, 9,500, with polymorphonuclear neutrophils, filamented,

49 per cent, nonfilamented, 13 per cent, eosinophils 4 per cent, lymphocytes 15 per cent, and monocytes 19 per cent; platelets, 360,000. The Wassermann and Kahn tests were negative. The urine was normal. The bengal rose dye excretion test showed 58 per cent retention in 8 minutes, and 30 per cent retention in 16 minutes. Echinococcus antigen and human and bovine tuberculin tests gave negative results. The dextrose tolerance test with insulin was normal.

The mass in the cervical region was removed surgically, and the pathologic diagnosis was cavernous lymphangioma (cystic hygroma). One month later splenectomy was performed. At this operation, exploration revealed that the liver and gallbladder were grossly normal, that both ovaries were present and that the left contained a cystic tumor, 5 cm. in diameter.

Pathologic Report.—The specimen consisted of a spleen weighing 2,120 Gm. and measuring 26 by 20 by 9 cm. It was deep reddish purple and studded with many cystic structures, varying from 0.5 to 4 cm. in diameter. Some of the cysts were tense, others fluctuant, and when the organ was sectioned, they were seen to contain a sticky, albuminous fluid which occasionally was hemorrhagic. The cysts were smooth lined, and some were multiloculated.

The greater part of each section consisted of small to rather large, frequently conglomerate, cystic structures, replacing and distorting a background of normal splenic tissue. Some of these spaces were empty; more of them contained a pale pink serum-like material. A few were filled with blood, but inasmuch as many spaces contained only serous fluid it is probable that the presence of blood was an artefact. The cysts were lined with a very flat endothelium, and just beneath this was a fibrous wall of variable thickness which merged with the adjacent splenic tissue.

Diagnosis.—Cystic lymphangioma of the spleen.

Case 3.—A 31 year old single white woman complained of soreness and swelling of the left upper quadrant of the abdomen of six years' duration. The family history and the patient's past history are irrelevant. The only history of trauma was that of a broken ankle which she had at 14. At 15 she had a spontaneous attack of "pleurisy" with pain of the left upper quadrant. Movement of any type produced a pain on her left side. On the advice of a physician she stayed in bed for two weeks, during which time her temperature rose to 101 to 102 F., and a friction rub was detected over the spleen. Since then she has had bouts of aching of the left shoulder and soreness of the left upper quadrant of the abdomen. Two years later another physician noted that her spleen was enlarged. She was treated medically, without relief.

The significant findings were limited to the abdomen. The liver was palpable and of normal size. A mass in the left upper quadrant, considered to be the spleen, extended 2 cm. to the right of the midline and 8 cm. below the left costal margin. It had a rounded edge, was not tender, had one prominent boss, and was thought to be attached to the diaphragm.

The hemoglobin was 90 per cent (12.3 Gm.); the blood counts were: red cells, 4,800,000 and white cells 7,900, with polymorphonuclear neutrophils 76, lymphocytes 16 and monocytes 8 per cent. The platelet count was 380,000. Red cell fragility was normal. The icterus index was 5. The bengal rose dye excretion test revealed 50 per cent retention in 8 minutes and 34 per cent retention in 16 minutes. The dextrose tolerance test with insulin gave a normal result. The Kahn test was negative.

The clinical diagnosis was: splenomegaly; possible congenital cystic disease of the spleen.

A high left rectus incision revealed a greatly enlarged spleen that was adherent to the abdominal wall. It was removed intact. Examination of the liver showed it to be normal. Postoperative recovery was entirely uneventful.

Pathologic Report.—The specimen consisted of a spleen weighing 1,640 Gm. and measuring 18 by 14 by 12 cm. One side of the organ appeared normal. At the lower pole the spleen merged into and formed part of the wall of a large single cyst, the interior of which was fairly smooth and contained turbid brown fluid exhibiting a shimmering sparkle due to cholesterol crystals. The wall was 3 to 6 mm. thick and was firm and fibrous.

There was no cellular layer lining the cyst wall, which was composed of dense connective tissue. The inner surface showed degeneration and scattered patches of adherent macrophages. In the cavity of the cyst, amorphous eosinophilic material and cholesterol crystals were encountered. Behind the dense, thick connective tissue wall the splenic pulp was rather fibrous. The malpighian bodies were not particularly prominent; the blood vessels were normal. No areas of deposition of iron pigment were seen.

Diagnosis.—Solitary secondary cyst of the spleen.

COMMENT

The incidence of secondary cysts of the spleen is about four times that of primary cysts. Tumors of angiomatous origin constitute about 65 per cent of the latter. The remainder comprises the epidermoid and dermoid cysts. The dermoid cyst is rare but easily identified. It must be at least partially lined with stratified squamous epithelium and reveal frank keratinization or epidermal appendages (e. g., hair or sebaceous or sweat glands). Such a dermoid cyst is exceedingly rare, only 2 instances having been reported (Kumaris [3] and Andral [4]). A third instance of possible splenic dermoid cyst, found in the mesentery at the splenic hilus, has been reported by Velasco Suarez and Angel Etcheverry.[5]

The identification of epidermoid cyst of the spleen is more difficult. It must be at least partially lined with stratified squamous epithelium. The difficulty of classification lies in determining whether the observed lining of squamous cells is true epithelium rather than transformed mesothelium or endothelium. The distinction cannot always be made. However, there may be present certain identifying characteristics, which should be evaluated in order to give accuracy to the reported incidence of true splenic cysts derived from epithelium.

The ontogeny of the spleen offers little aid in the problem, except to emphasize that true epithelium is rarely involved in the development of the spleen. In the early development of the mesogastrium, the splenic

3. Kumaris, J.: Arch. f. klin. Chir. **106**:699, 1915.

4. Andral, G.: Précis d'anatomie pathologique, Bruxelles, A. Wahlen et Cie, 1837, p. 432.

5. Velasco Suarez, C., and Angel Etcheverry, M.: Arch. argent. de enferm. d. ap. digest. y de la nutricion **12**:168, 1936-1937.

anlage is seen to arise from mesenchymal cells as well as from mesothelial cells of the overlying peritoneal surface. The contribution to the splenic parenchyma of the mesothelial peritoneum is rather limited, the major portion being derived from mesenchyme. Some of the early embryologists believed that portions of the spleen arose from cells given off by the nearby pancreatic epithelium, but the more recent work of Thiel and Downey [6] dismissed the possibility of an endodermal contribution to the development of the normal mammalian spleen. In regard of the wolffian bodies, they are spatially too remote to be considered as sources of cystic anomalies of the spleen, although Santy [7] has advanced such an hypothesis. Because of the prominence of mesenchymal cells in the normal development of the spleen, the proof that a primary tumor originated from epithelium must be given with great care, and must overshadow any possibility of the tumor's having been derived from mesenchyme.

An occasional cluster of multiple-layered cells is not a sufficiently distinguishing characteristic to differentiate between an epithelial and a mesothelial (or endothelial) origin of a cyst. This is especially true because much of the reported stratification occurs in crevices and recesses within the cyst wall, and these may be fortuitous. In regard to intercellular bridges, they are suggestive of ectoderm, but Heidenhain [8] reported their presence in mesodermal lining cells. If, in addition to stratification and the presence of intercellular bridges, keratinization occurs, the point in favor of a true epithelial origin becomes quite convincing. Lereboullet, Gregoire, Bernard and Ibarran [9] reported a "granular layer containing eleidin granules" in the cells of their case, but they have been the only ones to find even that suggestion of prekeratotic change. In regard to the significance of associated satellite cysts, their cytologic character should be a major contributing factor in deciding whether the observed stratified cellular lining of a cyst represents simple multiplicity of mesenchymal cell layers or layering of true epithelium.

In the first case reported here, the satellite cysts were clearly mesothelial in type, and it is presumed that the multilayered squamous cells arose from stratification of mesothelial cells. Such an origin without doubt accounts for most of the so-called "epidermoid" cysts. The term "epidermoid" as applied to splenic tumors is often interpreted as implying origin from epithelial precursors, and hence is inaccurate. The

6. Thiel, G. A., and Downey, Hal: Am. J. Anat. **28**:279, 1920-1921.

7. Santy, P.: Lyon chir. **27**:101, 1930.

8. Heidenhain, M.: Anat. Anz. **8**:404, 1893.

9. Lereboullet, P.; Gregoire, R.; Bernard, J., and Ibarran, R.: Sang **13**:853, 1939.

desirable term, although cumbersome, would be "metaplastic epidermoid mesodermal cysts" or, for brevity, metaplastic mesodermal cysts, thus leaving no doubt of their true origin.

Case 2, in which the patient suffered from cystic lymphangioma of the spleen, needs no further clarification in regard to the cellular origin of the tumor. It was interesting that there were angiomatous abnormalities in other tumors. The patient had had three large pelvic cysts removed, the origin and structure of which were not determined. In addition, a tumor diagnosed as lymphangiomatous hygroma was removed from her neck, and, finally on her left index finger she had a small hemorrhagic tumor which showed cyclic changes associated with the menstrual periods. These multiple tumors having similar backgrounds suggest that the tumor of the spleen was not a neoplasm in the strict sense, but another focus of a generalized congenital anomaly of abnormal vascular channels—hamartoma.

In case 3 there was revealed a typical example of a secondary cyst of the spleen. Before establishing such a diagnosis, however, one should make every effort to take sections of crevices and diverticula, which frequently show a characteristic lining cellular layer. There were none present in the cyst removed from this patient; instead, dense fibrosis of the wall and a lumen filled with brown sediment containing cholesterol were found. Much of the debris was the residue from old hemorrhages of the cyst.

SUMMARY

Over a period of five years, 3 patients with enlargement of the spleen were observed, and in each was found a different type of nonparasitic, noncancerous cystic tumor. A large mass in the left upper quadrant of the abdomen directed attention to the spleen, and complete examination supported the original clinical impression but was insufficient to establish an absolute diagnosis. There were no characteristic hematologic findings. The following tumors were removed:

1. An "epidermoid" cyst, better termed a metaplastic epidermoid mesodermal cyst. The literature on the subject is reviewed, and the embryologic derivation of these tumors is discussed. Custom has led to the false labeling of these cysts as "epidermoid" cysts. It is suggested that the term "metaplastic mesodermal cysts" would be embryologically and developmentally the most accurate term for them.

2. A diffuse cystic lymphangioma, which was attended by other angiomatous tumors elsewhere.

3. A simple secondary cyst of the spleen.

NUCLEAR SIZE IN TETANIZED AND IN CURARIZED SKELETAL MUSCLE

RUDOLF ALTSCHUL, M.U.Dr.
SASKATOON, SASKATCHEWAN, CANADA

IN PREVOUS publications [1] I expressed the view that decrease of interstitial pressure may be responsible for an increase of nuclear size and in this way may initiate amitotic division, according to the fact that nuclei divide when they reach a certain size ("critical phase"). My view was based on the reactions of nuclei observed in denervated or mechanically injured skeletal muscle, in amputation neuromas, in nerve transplants, in tissue cultures of nerves and in severed tendons.

In the case of skeletal muscle it appears that it is the wasting of sarcoplasm which upsets the pressure equilibrium in the muscle fiber, leading to a relative increase of intranuclear pressure. The cutting of perineurium and endoneurium elicits in most cases an enlargement of the nuclei of Schwann cells and of endoneurial fibroblasts. However, if prior to the injury the nerves are tightly ligated proximally, the severance is not followed by an increase in nuclear volume. It is probable that the maintenance of interstitial pressure prevents enlargement of the nuclei. Release of tension by severing tendons frequently causes shortening and widening of the tendon cell nuclei with increase of volume. What secondary changes are responsible for the nuclear enlargement is not yet known. There may occur an alteration in the permeability of the nuclear membrane and changes by osmosis and imbibition.

As already mentioned (1948), securing direct proof for the primary role of changes of tissue pressure appears difficult if not impossible, and it was suggested that variations of the previous experiments [1] might give additional support for the thesis. In the present report two new approaches are described.

MATERIAL AND METHODS

The animals used in these experiments were frogs (rana pipiens) and white rats. The subsarcolemmal nuclei of skeletal muscle are large in frogs; they become only slightly contorted (or not contorted at all) if the muscle is tetanized. In the first

From the Department of Anatomy, University of Saskatchewan.

This study was supported by a grant from the Division of Medical Research of the National Research Council of Canada.

1. Altschul, R.: Arch. Path. **34**:982, 1942; Rev. canad. de biol. **6**:485, 1947; Anat. Rec. **100**:517, 1948.

TABLE 1.—*Nuclear Size in Tetanized Muscle*

	Normal Muscle *							Tetanized Muscle *							
Number	Width	Standard Deviation	Standard Error	Length	Standard Deviation	Standard Error	Volume	Width	Standard Deviation	Standard Error	Length	Standard Deviation	Standard Error	Volume	Per Cent
1	8.282	.981	±.221	19.43	2.988	±.668	697.4	8.264	.716	±.160	16.75	2.289	±.512	598.6	−14
2	8.503	.796	±.178	19.21	2.837	±.634	726.8	8.733	.919	±.206	15.52	2.263	±.506	619.4	−15
3	8.141	.601	±.134	20.15	2.457	±.549	698.9	8.618	.804	±.180	15.27	2.395	±.536	593.5	−15
4	7.425	.714	±.130	19.86	3.306	±.604	573.0	7.699	1.096	±.200	16.42	2.687	±.491	509.3	−11
5	8.742	.610	±.136	17.78	2.661	±.595	711.1	8.795	.751	±.168	16.89	2.537	±.567	683.7	−3.9
6	8.503	.849	±.155	19.37	2.722	±.497	732.9	7.920	1.114	±.203	16.23	3.085	±.563	532.7	−27
7	8.167	.804	±.147	19.05	2.793	±.510	664.9	8.097	.725	±.132	16.04	2.316	±.423	550.3	−17
8	8.052	.893	±.163	16.57	2.475	±.452	562.2	7.637	.734	±.134	15.84	2.943	±.537	483.5	−14

* All measurements are given in microns.

TABLE 2.—*Nuclear Size in Curarized Muscle*

	Normal Muscle *							Curarized Muscle *							
Number †	Width	Standard Deviation	Standard Error	Length	Standard Deviation	Standard Error	Volume	Width	Standard Deviation	Standard Error	Length	Standard Deviation	Standard Error	Volume	Per Cent
9	8.132	.831	±.186	18.44	2.616	±.585	638.1	8.503	.734	±.164	21.48	2.139	±.478	812.7	27
10	8.194	.787	±.176	19.99	2.395	±.536	702.4	8.459	.698	±.156	22.02	2.325	±.520	824.5	17
11	8.105	.707	±.158	20.44	2.722	±.609	702.6	8.715	.689	±.154	21.24	2.236	±.500	844.2	20
12	7.919	.672	±.106	20.91	3.589	±.567	686.2	8.07	.831	±.131	21.76	3.787	±.599	741.6	8
13	8.097	.925	±.146	22.17	3.438	±.544	760.6	8.23	.799	±.127	21.36	3.129	±.495	757.1	−0.5
14	8.282	.571	±.128	20.06	3.038	±.679	720.0	8.671	.858	±.192	21.21	2.306	±.516	834.5	16
15	7.876	.711	±.112	19.86	5.109	±.808	644.7	8.299	.919	±.145	22.04	3.615	±.572	794.4	23
16	7.584	.653	±.119	21.95	3.366	±.615	660.7	8.017	.902	±.165	22.72	3.369	±.615	764.2	16

* All measurements are given in microns.
† Nos. 9 to 11 were curarized by intramuscular and intracardial injection; nos. 12 to 16, by intramuscular injection only.

series, of experiments the brain of the animal was destroyed and then the gastrocnemii were excised. One was fixed immediately in 10 per cent formaldehyde solution, while the other was tetanized by faradic current and immersed in 10 per cent formaldehyde solution, the faradization being continued while the muscle was being fixed, until no more relaxation or contraction could be obtained.

In the second series, the gastrocnemius of one leg of the frog in which the brain had been destroyed was excised and fixed in 10 per cent formaldehyde solution; then the animal was curarized by injecting "intocostrin" (0.75 to 0.8 cc. of the solution) intramuscularly and intracardially. When the animal was completely paralyzed, the gastrocnemius of the other leg was dissected out and fixed in 10 per cent formaldehyde solution. All the muscles were embedded in paraffin, cut longitudinally and stained with hematoxylin and eosin.

Similar experiments were also carried out on rat muscles. While the tetanizing yielded results conforming with those of the frog experiments, the curarizing of rats was not successful, for small doses gave incomplete relaxation of muscles and large doses elicited fasciculation. For this reason the curare experiments with rats were abandoned.

In measuring the nuclei, the following procedure was followed: the width and the length of each of 20, 30 or 40 nuclei were determined from each muscle, the widest nuclei being selected for the measurements. This was done with the view that (1) if the widest nuclei were selected, the error of a perspective shortening of the long axis would be of lesser importance; (2) one would be dealing with a more homogeneous group; (3) the wide nuclei being by far the more voluminous, the margin of error might be expected to be smaller. In control measurements nuclei were taken at random, i.e., without regard to their width. In some cases 100 nuclei instead of only 20 to 40 were measured. All measurements were done by one person (Miss A. M. Friesen) using oil immersion and an ocular micrometer. Then the average length and the average width of the nuclei were determined, and the average volume ($4/3\pi ab^2$) established—assuming that these nuclei are spheroids. For control purposes, we established also in a few cases (1) the volume of each nucleus and then the average volume, as well as (2) the average width and length and from them the average volume. The latter was practically identical with that obtained by the first procedure.

RESULTS AND COMMENT

The results of the frog experiments are evident from tables 1 and 2. The decrease of tonus and the nuclear enlargement in curarized muscle conform with the findings in denervated muscle. In 5 rats the tetanizing of muscle and the increase of tonus resulted in a decrease of volume in subsarcolemmal muscle nuclei by 4, 4, 5, 17 and 19 per cent, respectively.

Admittedly, the technic involves one major error, namely, the assumption that the cross section of a nucleus is a perfect circle, which it never is and which it rarely approaches. But in injured peripheral nerves (Abercrombie and Johnson [2]; Denny-Brown [3]), in skeletal muscle where the sarcolemma was ruptured (Barer [4]) and in curarized

2. Abercrombie, M., and Johnson, M. L.: J. Anat. **80**:37, 1946.
3. Denny-Brown, D.: Arch. Neurol. & Psychiat. **55**:171, 1946.
4. Barer, R.: J. Anat. **81**:259, 1947.

skeletal muscle (my own observations) the cross section of the nucleus becomes rounder as compared with the nucleus of normal nerve or muscle. Therefore the error in assuming that the cross section is a circle in both the normal and the experimental conditions will influence the results by decreasing and not by creating or even increasing the difference between the muscles of the two sides. That means that the differences are most probably greater than reported in table 2 and in a previous publication (1948), and possibly also greater than in table 1. It could be said that from a statistical point of view the number of measured nuclei is small. This may be true, but it should be pointed out that similar measurements were carried out in 52 other cases, published previously and some more, hitherto unpublished, and in all these cases the conditions of decrease or maintenance of interstitial pressure led to similar results regarding the volume of the nucleus. Moreover, nuclear enlargement has been reported under similar conditions, but without measurements and without regard to tissue pressure by Weber [5] and many others for nuclei of skeletal muscle, and by Masson [6] and by Abercrombie and Johnson [2] for sheath cell nuclei of nerves.

SUMMARY

The thesis that changes of extranuclear pressure are followed by changes in nuclear volume receives additional support in two sets of experiments: In the first, muscle tonus was increased by tetanizing and the nuclear volume decreased accordingly; in the second series the volume of nuclei of skeletal muscle increased when muscle tonus decreased under curarizing. The latter results parallel those of muscle denervation.

5. Weber, O.: Virchows Arch. f. path. Anat. **39**:216, 1867.
6. Masson, P.: Am. J. Path. **8**:367, 1932.

ORGANOID DIFFERENTIATION OF THE FETAL LUNG

A Histologic Study of the Differentiation of Mammalian Fetal Lung in Utero and in Transplants

WILLIAM R. WADDELL, M.D.*
BOSTON

FOR MORE than one hundred years controversy regarding the ultimate structure of the pulmonary alveolus has waxed and waned. One of the principal issues of disagreement has been the question of whether or not this structure is lined by epithelium. Since an understanding of the genesis of various congenital, inflammatory and neoplastic pulmonary lesions depends on the establishment of this and other facts relating to the normal alveolus, the problem is of more than academic interest.

The older literature on the subject was reviewed by Miller,[1] who favored the concept of a continuous alveolar lining of epithelial cells. Other investigators[2] concluded that the cells which proliferate and line the alveoli in response to injury or to the presence of foreign material are derived from the macrophage system. Still others[3] hypothecate the presence of an alveolar lining comprised of non-nucleated protoplasmic plates or films. Rose[4] enlivened the controversy still further by asserting that he could find no evidence that the alveoli develop as an outgrowth of the bronchial tree. He studied the formation of the alveolar capillary plexus from the mesoderm and regarded it as the essential anatomic and functional unit of the lung, bearing a relationship to the bronchi similar to that borne by the glomerular capillaries to the nephron.

---

*Research Fellow in Legal Medicine, Harvard Medical School.

This work has been aided in part by a grant from U. S. Public Health Service.

1. Miller, W. S.: The Lung, Springfield, Ill., Charles C Thomas, Publisher, 1937.

2. Fried, B. M.: Arch. Path. **3**:751, 1927; **6**:1008, 1928. Robertson, O. H.: Psysiol. Rev. **21**:112, 1941. Geever, E. F.; Neubuerger, K. T., and David, C. L.: Am. J. Path. **19**:913, 1943. Clements, L. P.: Anat. Rec. **78**:429, 1940. Marshall, A. H. E.: J. Path. & Bact. **108**:129, 1947.

3. Bensley, R. D., and Bensley, S. H.: Anat. Rec. **64**:41, 1935. Bensley, S. H., and Groff, M. B.: ibid. **64**:27, 1935. Cooper, E. R. A.: J. Path. & Bact. **47**:105, 1938. Stewart, F. W.: Anat. Rec. **25**:181, 1923. Bremer, J. L.: ibid. **70**:263, 1938.

4. Rose, S. B.: Arch. Path. **6**:36, 1928.

Palmer,[5] Barnard and Day[6] and Ham and Baldwin[7] have more recently emphasized the participation of the capillaries in the development of the terminal air passages as observed in the human fetus. Their studies indicate that the cells which line the terminal vesicles of the immature lung begin to disappear about the end of the fifth month of fetal life and that the unsheathed capillaries come in direct contact with the alveolar space.

The investigation of this problem has been carried on in the past by microscopic examination of serial sections of the lungs of embryos of increasing age. Such sections have usually been stained with hematoxylin and eosin or prepared by comparable nonspecific procedures. There have been numerous exhaustive analyses of such material from several species, some leading to one conclusion, some to another. Maximow and Bloom[8] have summarized the matter with the statement that "the whole question of the type of cell lining the alveoli and the determination of its epithelial or mesenchymal origin demands a thorough embryologic investigation of the lungs in the later stages of intra-uterine life."

With this background there would appear to be little encouragement for one to reinvestigate the problem by the same methods that have been employed in the past. It was felt, however, that there were at least two promising approaches that had not been adequately explored. One was to investigate the cytochemical characteristics of the differentiating pulmonary blastema in the belief that structural differentiation of primitive cells may be preceded by chemical change. Recognition of such change preparatory to structural differentiation might help to establish whether the epithelial elements of the bronchi and the alveoli are derived from the foregut by an infiltrative proliferation of endodermal cells or whether they represent in situ metaplasia of the cells of primitive pulmonary mesenchyme.

Another investigative procedure considered worthy of trial was to employ the method which Greene[9] recently described, by which fetal tissue may be caused to grow and differentiate in a heterotopic environment by transplanting it to the anterior chamber of the eye. In these circumstances, organoid differentiation of fetal tissue takes place independently of the influences of somatic integrity, and any differentiation that occurs must be inherent in the transplanted cells themselves and independent of the extrapulmonary organizers or gradients that affect organoid differentiation in the intact fetus.

---

5. Palmer, D. M.: Am. J. Anat. **58**:59, 1936.
6. Barnard, W. G., and Day, T. D.: J. Path. & Bact. **45**:67, 1937.
7. Ham, A. W., and Baldwin, K. W.: Anat. Rec. **81**:363, 1941.
8. Maximow, A. A., and Bloom, W.: Text-Book of Histology, ed. 4, Philadelphia, W. B. Saunders Company, 1943.
9. Greene, H. S. N.: Cancer Research **3**:809, 1943.

HISTOCHEMICAL INVESTIGATION

In the course of surveying the cytochemical characteristics of fetal lung in respect to the presence or the distribution of nucleoprotein (Feulgen reaction [10]), mucoprotein,[11] fat (sudan IV),[12] alkaline phosphatase [13] and glycogen [14] it became apparent that the appearance and disappearance of intracellular glycogen bear an important relation to alterations of cellular appearance and organization.

Uninterrupted series of sections stained with Best's carmine were cut from blocks of fetal lungs (mouse, guinea pig, human) of varying gestational age that had been fixed in Carnoy's or Rossman's fluid. Similar series of sections were cut from blocks of the same lungs that had been fixed in Zenker's fluid or 10 per cent formaldhyde solution and stained with either hematoxylin and eosin or eosin–methylene blue.

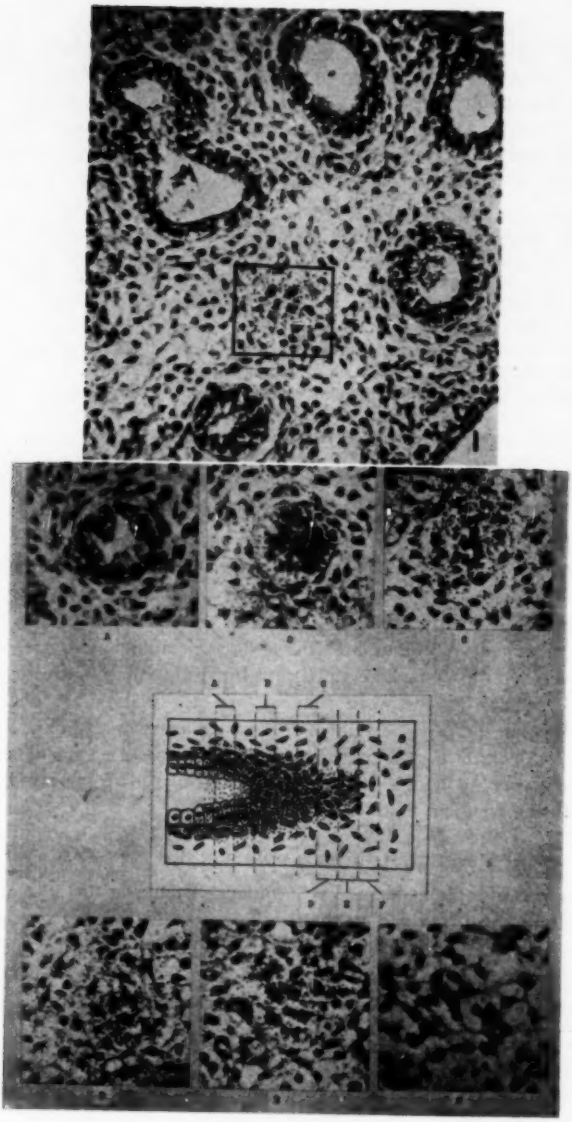
There were no fundamental differences in these three species of mammals in respect to the histologic appearance of, or relationships between, the elongating and branching bronchi and the primitive mesenchyme in which organoid development of lungs takes place. No fetus was studied which was so young that the pulmonary blastema was devoid of recognizable bronchial differentiation. Fetuses obtained in the first trimester of pregnancy were most suitable for the study of bronchial development whereas those obtained in the second and third trimesters were used for the investigation of alveolar differentiation.

In fetuses so young that there was as yet no alveolar differentiation the primitive lung was comprised of a branched epithelium-lined tube situated in a solid mass of undifferentiated mesenchyme. In the youngest fetuses the mesenchyme was relatively avascular and composed of loosely arranged undifferentiated tissue having an abundant intercellular fluid except in the immediate vicinity of the differentiating alveoli where the intercellular spaces were reduced and the cells more compactly arranged (fig. 9). As the pulmonary blastema of slightly older fetuses became vascularized, the entire mesenchyme became more compact and the looser structure seen in younger embryos disappeared along with the intercellular fluid (fig. 10).

BRONCHIAL DIFFERENTIATION IN UTERO

In series of sections stained by ordinary visualizing methods (hematoxylin and eosin or eosin–methylene blue) the impression was gained that the peripheral extension of the primitive bronchi was accomplished by multiplication of the most distally located epithelial cells which invaded and replaced the adjacent mesenchymal elements (figs. 9 and

10. Feulgen, R., and Rossenbeck, H.: Ztschr. f. physiol. Chem. **135**:203, 1924.
11. Hemplemann, L. H., Jr.: Anat. Rec. **78**:197, 1940.
12. McClung, C. E.: Handbook of Microscopial Technique, New York, Paul B. Hoeber, Inc., 1937.
13. Gomori, G.: J. Cell. & Comp. Physiol. **17**:71, 1941.
14. Bensley, C. M.: Stain Technol. **14**:47, 1939.



Figures 1 and 2.

10). Thus, it has often been inferred from the study of such preparations that the parenchymatous elements of the pulmonary tissue or at least the bronchial epithelial cells are the direct descendants of the endoderm of the foregut.

In series of sections cut from blocks fixed in Carnoy's or Rossman's fluid and stained by the Best carmine technic it became apparent that the bronchial epithelium is probably derived by a quite different process. Peripheral to the growing tip of a bronchus and in continuity with it, a chemical alteration occurs in the primitive cells of the mesenchyme which is the first of a series of changes by which the mesodermal cells are transformed into bronchial epithelium. This chemical change is antecedent to any recognizable alteration of their size, shape or relationship to one another. It consists of an intracytoplasmic accumulation of small droplets of glycogen. These droplets are so small that without special preparation and staining they escape detection; i. e., they are not large enough to give the appearance of vacuolation after they have been removed by solution (figs. 2 and 9). This preliminary chemical change which appears to be a precursor of structural differentiation is not peculiar to the transformation by which mesenchyme becomes bronchial structure. It also precedes the metamorphosis by which mesoderm develops as primitive blood vessels (fig. 3).

In the case of developing bronchi the glycogen appeared in the cytoplasm of the mesenchymal cells as far as 20 or 30 microns in advance of recognizable structural differentiation (fig. 2 *C, D* and *E*). Immediately proximal to this initial chemical alteration the first morphologic evidence of differentiation appeared (fig. 2, *B* and *C*). The glycogen-containing cells underwent active mitotic division, became individually swollen and formed a poorly defined but compact cellular island in the otherwise loosely arranged mesenchyme. As the tip of the elongating

Fig. 1.—Low magnification orienting view of a portion of the lung of a 44 mm. guinea pig embryo. In the enclosed area the fine stippling is due to small intracytoplasmic accumulations of glycogen distal to the recognizable tip of a bronchus. Other than this chemical differentiation there is no evidence that this area is proceeding toward bronchus formation. The five larger bronchi show accumulations of glycogen, and in two of them there are small quantities of glycogen which have been extruded into the lumens. The schematic reconstruction of the bronchus shown in figure 2 was made from serial sections passing through this area. Best carmine and hematoxylin; × 225.

Fig. 2.—Semidiagrammatic reconstruction of the tip of a differentiating bronchus of the lung of a 44 mm. guinea pig embryo fixed in Carnoy's fluid and stained for glycogen by the Best carmine method. Sections *A, B* and *C* are 10 microns apart; *D, E* and *F* are 5 microns apart. Glycogen is demonstrated in the photomicrographs as darkly stained intracytoplasmic accumulations. It is correspondingly represented by black stippling in the diagram. *C, D* and *E* pass through the zone of chemical differentiation described in the text. There is no morphologic evidence of differentiation here. *B* shows central crowding of nuclei as well as large central accumulations of glycogen. This is the most distal morphologically recognizable part of the bronchus. *A* demonstrates a well formed bronchus with definite epithelium and only a few glycogen droplets. The nuclei are nearer the periphery. Best carmine and hematoxylin; × 870.

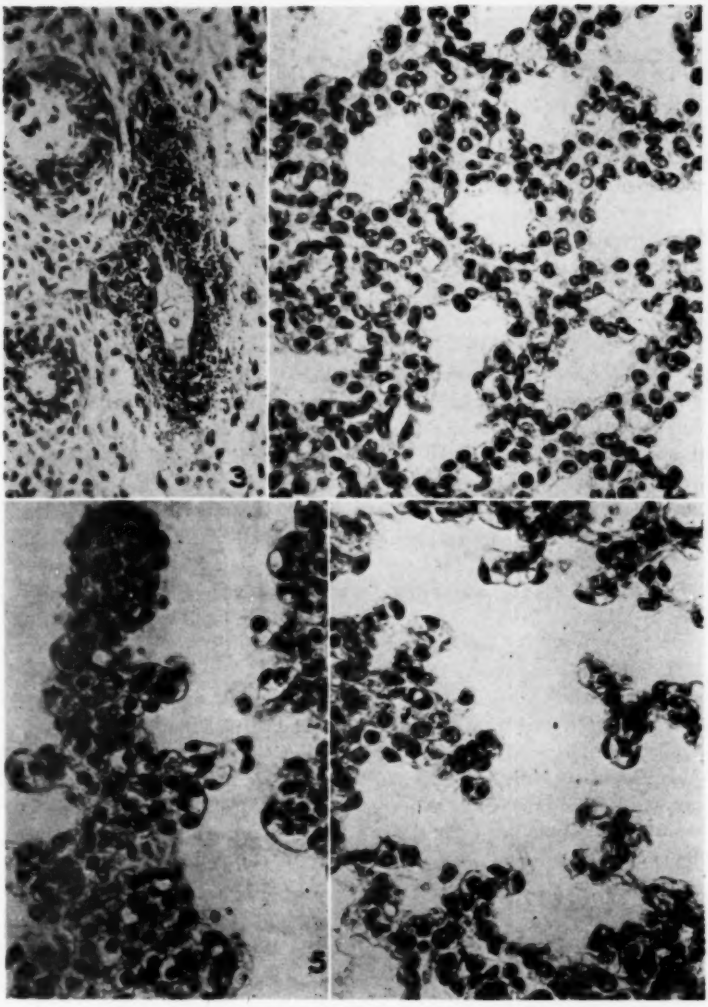


Fig. 3.—Glycogen accumulating in and about a differentiating arterial wall in the lung of a 44 mm. guinea pig embryo. Glycogen is also present in the bronchial epithelium. Best carmine and hematoxylin; × 250.

Fig. 4.—An early stage of development of a terminal respiratory unit in an 80 mm. guinea pig embryo. The parenchyma is still compact, and scattered through it can be seen blood vessels which, although near the lumens, have not come in close contact with them. Notice that not all the cavities have definite epithelial linings. The alining of the mesenchymal cells bordering on these cavities to form epithelium without a basement membrane can be seen. Hematoxylin and eosin; × 425.

Fig. 5.—Early alveolar formation in the lung of a 95 mm. guinea pig embryo. The parenchyma has thinned out and capillary knuckles are pushing into the lumens. Hematoxylin and eosin; × 425.

Fig. 6.—Further capillary development in the lung of a 105 mm. guinea pig embryo. The bulk of the undifferentiated mesenchyma has disappeared, and the number of capillaries has increased. The only visible structure separating the alveolar space from the blood stream is the capillary endothelium. Hematoxylin and eosin; × 425.

bronchus was approached the central cells in such a cluster took on an ill defined radial arrangement and the cytoplasmic inclusions of glycogen increased to such a degree that the cells became coarsely vacuolated (fig. 2 *C*). As yet there was nothing to distinguish these altered mesenchymal cells from their fellows or to indicate that they were to become bronchial epithelium aside from their radial arrangement, their swelling and the increase of their glycogen content. As these cells merged with those of the solid tip of the bronchus, the glycogen-containing vacuoles at their outer poles became very large and the nuclei were crowded into a central cluster. This resulted in an apparent but not actual local increase in the number of cells (fig. 2 *B*). As observation moved still further into the distal end of what was now recognized as a bronchus, disintegration of some of the central cells was noted. The survival of a single peripheral layer which had compressed the surrounding mesenchyme resulted in the formation of a basement membrane and completed the formation of the epithelium-lined bronchial tube (fig. 2 *A*).

The fact that in the rapidly growing fetal lung mitotic activity was most pronounced not at the tips of the elongating bronchi but in the undifferentiated mesenchyme peripheral to them has already been mentioned but deserves further emphasis. If the elongation of bronchi resulted from the multiplication of cells of endodermal origin one would expect the tip of the growing bronchus to be the site of greatest mitotic activity as is the tip of a growing root of a plant.

When glycogen first appeared, it was uniformly distributed in the cytoplasm of the activated mesenchymal cells. As this chemical precursor of structural differentiation proceeded, the glycogen tended to accumulate in the peripheral end of differentiating epithelial cells but gradually shifted to the luminal or central ends of cells, where it persisted for a short time and was finally extruded into the lumen of the primitive bronchus. Following the extrusion of the glycogen droplets, the cells became cuboidal, and their nuclei moved peripherally toward the basal ends of the cells that were now unmistakably epithelial in character. The glycogen lying free in the lumen gradually disappeared, and by the third trimester of fetal life only small traces remained.

ALVEOLAR DIFFERENTIATION IN UTERO

The process described in the previous paragraphs was the dominant feature of the development of the lungs during the first trimester of fetal life and led to the branching and elongation of bronchi which was preparatory to alveolar differentiation. Not until the second and third trimesters of fetal development did alveolar differentiation become the dominant developmental change. In contrast to the consolidation

and enlargement of mesenchymal cells which preceded bronchial differentiation, the development of the pulmonary alveolus was initiated by rarefaction. In preparation for alveolar development the mesenchymal cells at the tips of terminal bronchi became peripherally displaced as though by fluid. Clusters of cavities thus formed communicated with the terminal bronchial lumen. Subsequently the mesenchymal cells lining such cavities enlarged as glycogen appeared in their cytoplasm. With this enlargement they became cuboidal and took on the appearance of epithelium. There was considerable species variation in respect to the time at which the innermost mesenchymal cells that had been displaced to form these primitive alveoli acquired the appearance of epithelium. In the mouse there was a relatively long lag between the preliminary cavitation of the mesenchyma and the epithelial differentiation of the lining cells. In the guinea pig and in man epithelization followed closely after the appearance of the cavities.

The presence of a continuous epithelial lining of the immature alveoli was transitory, and about the time that alveolar formation was nearing completion the enlarged lining cells discharged their cytoplasmic accumulations of glycogen. As the glycogen was shed into the alveolar lumen, some of the lining cells disintegrated in situ (fig. 7) and others desquamated (fig. 8) as though they were being dislodged by the rapidly growing capillary loops which by this time were pushing up through and between them (figs. 4, 5 and 6). Still others shrank and regressed to form the inconspicuous cells with small nuclei and scanty cytoplasm that have been variously referred to as septal cells (Lang [15]), epicytes (Clara [16]) or fixed macrophages (Fried [2]) and which are regarded by some as being of endodermal origin.

It would seem better to regard them as sensitive and plastic mesenchymal cells which are inactive and inconspicuous under normal conditions but which are capable of becoming active phagocytes when stimulated by the presence of foreign material and which may proliferate to form a continuous lining membrane in response to injury or may engage in more extravagant and atypical growth in such conditions as pulmonary adenomatosis or neoplasia.

DIFFERENTIATION OF FETAL LUNG TISSUE IN THE ANTERIOR OCULAR CHAMBER

Fetal lung tissues derived from guinea pigs, mice and rabbits were transferred to the anterior ocular chambers of adult guinea pigs by the technic [9] described by Dr. H. S. N. Greene, of New Haven, Conn.

15. Lang, F. J.: J Infect. Dis. **37**:430, 1925.
16. Clara, M.: Ztschr. f mikr.-anat. Forsch. **40**:147, 1936.

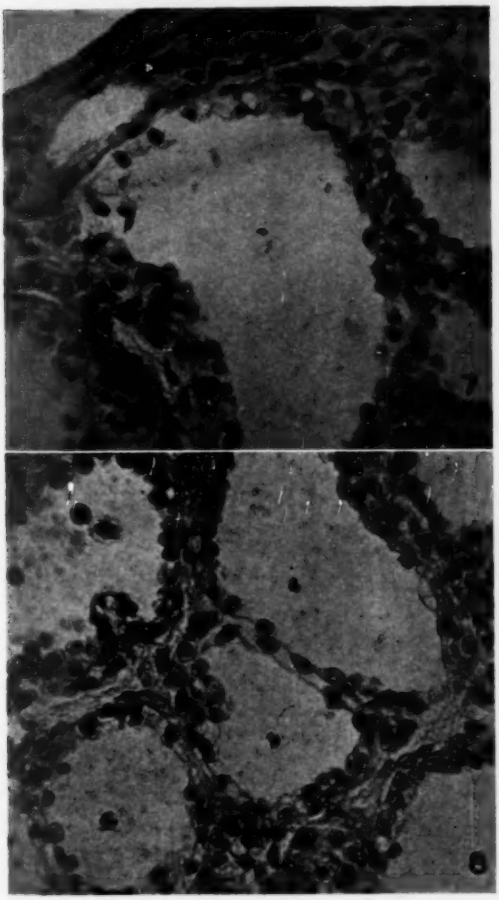


Fig. 7.—An alveolus of fetal guinea pig lung tissue that eight days earlier was transplanted from a 40 mm. guinea pig embryo to the anterior chamber of an adult guinea pig's eye. On the upper left margin is the capsule of the transplant corresponding to pleura. Epithelial lining that is in the process of degenerating can be seen. The clear vacuoles contained glycogen, the extrusion of which accompanies the disintegration of some of the lining cells. Hematoxylin and eosin; × 425.

Fig. 8.—Alveoli of the same 8 day old transplant shown in figure 7, in which can be seen the manner in which some lining cells become dislodged into the lumens as capillary development progresses. Notice the bare capillary separating two alveoli: Hematoxylin and eosin; × 425.

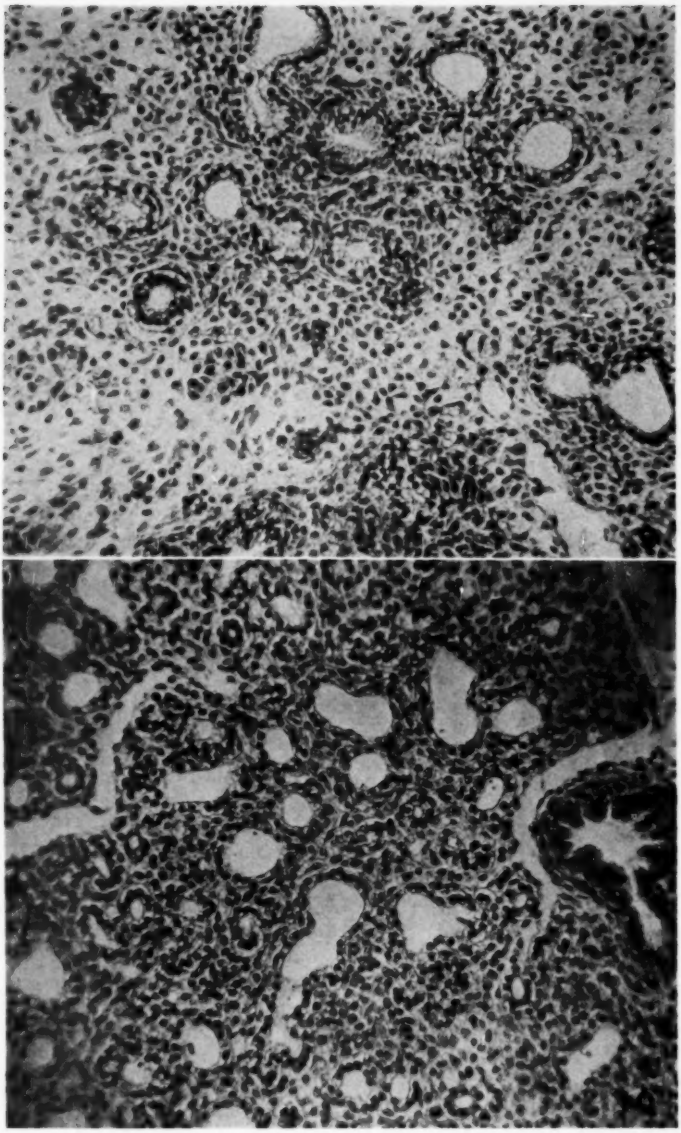


Fig. 9.—Lung of a 40 mm. guinea pig embryo. At this stage most of the primitive lung is composed of an undifferentiated mesenchyme having an abundant amount of intercellular fluid except in the immediate vicinity of the differentiating bronchi, where the intercellular spaces are reduced and the cells more compactly arranged. Hematoxylin and eosin; × 225.

Fig. 10.—Lung of a 54 mm. guinea pig embryo showing the results of an additional ten days' differentiation in utero. The lung is now comprised principally of small bronchi lined by cuboidal cells and separated from one another by compact mesenchyme. The loosely arranged mesenchyme composed of spindle cells seen in the 40 mm. fetus has almost completely disappeared. Hematoxylin and eosin; × 225.

Dr. Greene contributed assistance and advice in the development of this phase of the investigation. I had not tried this particular experimental technic before undertaking the investigation herewith reported, and many of the initial attempts to secure heterotopic growth of lung tissue were unsuccessful. With increasing experience it was found that the majority of such attempts were successful in that the transplanted lung tissue remained viable, underwent a transplantation phase of growth and differentiation and remained free of infection.

Survival and growth of transplants did not appear to be influenced by the strain, age or sex of the host animals. A fetus to be used as a source of lung tissue was removed by hysterotomy, with the animal under "pentothal sodium" anesthesia. When the uterus contained more than one fetus, one was taken for transplantation experiments and the others were left to develop in utero so as to serve as litter mate controls. Usually the controls were removed at the time that the transplants were excised for microscopic study. Although hysterotomy was frequently followed by abortion when performed during the terminal period of pregnancy, it rarely caused abortion when performed during the first trimester.

The observations reported in the succeeding paragraphs are derived from 38 transplants of lung tissue from guinea pig fetuses having crown-rump measurements ranging between 24 and 95 mm. The usual procedure was to divide the lung of the donor fetus into between four and six fragments of approximately similar size and shape. One of these was immediately fixed for control study and 1 to 2 mm. fragments of the others were transplanted to anterior ocular chambers. The transplants were removed for microscopic study after periods of heterotopic survival ranging between four and ninety days.

In general the incidence of successful translants bore an inverse relationship to the age of the donating fetus. In experienced hands the complete failure of a transplant derived from a fetus during the first or second trimester of gestational development to survive was unusual. The majority of such transplants not only survived but underwent a period of active growth, usually filling the anterior chamber. Although the majority of transplants from older fetuses remained viable and usually survived for weeks before atrophy could be perceived by clinical examination, active growth of such tissue was the exception rather than the rule.

After being introduced into the anterior chamber the transplanted fragment usually remained relatively unchanged for a period of between twelve and thirty-six hours. By the end of thirty-six hours the transplant was characteristically pink and visibly vascularized. New blood

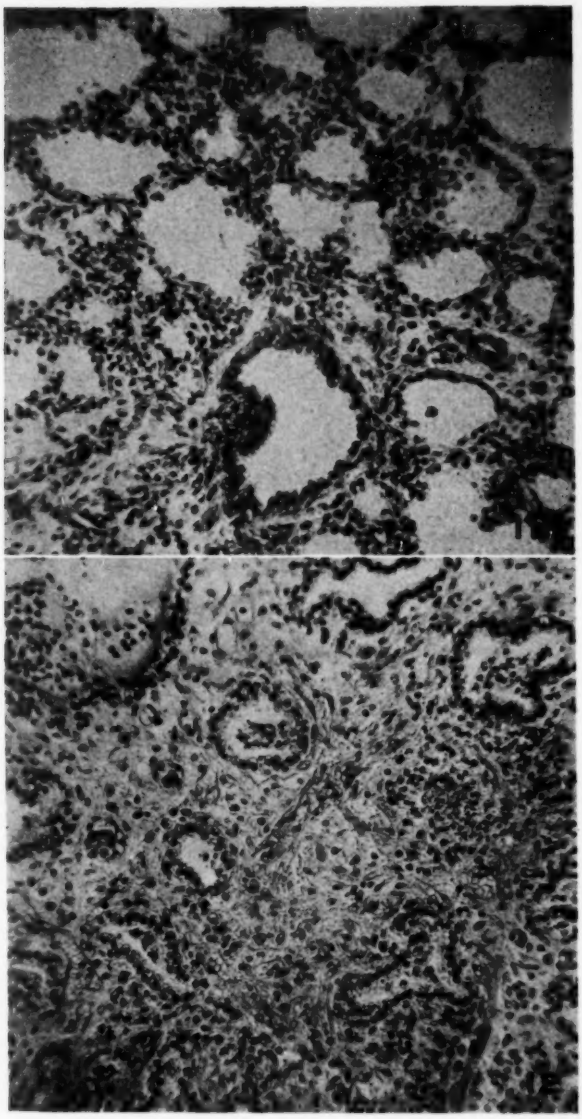


Fig. 11.—Eight day old transplant of fetal guinea pig lung. This tissue was transplanted from a 40 mm. embryo to the anterior chamber of an adult guinea pig's eye. The differentiation of this transplant has been greatly accelerated, and the degree of differentiation shown would not have been attained short of near-gestational maturity (about seventy-two days) in utero. Well developed alveolar structure and bronchi are shown. Most of the alveoli are lined by low cuboidal epithelium, but in some bare capillaries can be seen. Hematoxylin and eosin; × 225.

vessels extending between the iris or the cornea and the transplant were frequently visible by the end of the third day.

Behavior of Fetal Lung Tissue Transplanted in the Anterior Chamber of an Adult Guinea Pig's Eye.—Microscopic examination of fetal lung tissue after varying periods of heterotopic growth disclosed a sequence of four more or less well defined phases.

During the initial post-transplantation phase, which usually lasted for twelve to thirty-six hours, the transplant showed little or no change. A mild inflammatory reaction consisting of edema and hyperemia was elicited in the adjacent host tissue. Although failure to acquire a blood supply during this period usually resulted in degeneration and necrosis, an occasional transplant remained dormant for many days before growing or showing evidence of having acquired a blood supply from the host.

If, as was usually the case, the transplant acquired an adequate blood supply during the first few days, active proliferation occurred and continued for days or weeks. This proliferation took any one of several different forms. In some instances growth and differentiation took place in an orderly fashion and, although it often occurred either more or less rapidly than would have been the case if it had been taking place in the intact fetus, resulted in well differentiated alveoli and air passages with little or no residuum of undifferentiated mesoderm (fig. 11).

In other instances the proliferative phase was represented by multiplication of the mesoderm of the primitive blastema with little or no evidence of differentiation (fig. 13). The latter type of growth resulted in a cellular mass of tissue sometimes having a microscopic appearance remarkably similar to that of a rapidly growing spindle cell tumor. In transplants of this type there appeared to be actual dedifferentiation of many partially differentiated cells to a more primitive form. In some transplants only the most highly differentiated elements, such as the cartilage and the epithelium of the larger bronchi, remained to distinguish the original character of the tissue (fig. 12). In still others proliferation proceeded nonuniformly and atypically, leading to relatively normal organoid differentiation in some places and to an over-

Fig. 12.—Eleven day old transplant of fetal guinea pig lung. This tissue was transferred from a 40 mm. embryo to the anterior chamber of an eye of an adult guinea pig. The interstitial tissue that normally appears as in figures 1 and 2 has largely disappeared, being replaced by an edematous, loose fibrillar connective tissue. The remaining cells are disintegrating. The cytoplasm stains poorly. The nuclei are pyknotic. In some areas the spindle-shaped cells indicate that local tissue is being transformed to fibrous tissue. The more differentiated bronchial epithelium shows less evidence of degeneration. There is no reason to believe that this transplanted fetal tissue ever differentiated as did that shown in figure 11. Hematoxylin and eosin; × 225.

growth of the mesoderm of the primitive pulmonary blastema in others. Between these two extremes, all manner of abnormal or unbalanced growth took place.

After the initial period of active proliferation the transplant entered a static phase in which there was neither growth nor perceptible retrogression. This often lasted for days or weeks before passing on into the next and final phase of the life of the transplant.

The last phase in the sequence was one of involutional fibrosis and atrophy. The cicatrix, which was at first loosely cellular and edematous (figs. 12 and 14), eventually became dense and contracted.

The behavior of the individual components of such transplants was often exceedingly complex. It was difficult or impossible to draw any inferences as to the effect which the heterotopic environment had on pulmonary differentiation from a study of a single or, for that matter, of several individual transplants. It was only through a study of many transplants of varying age and by comparing one with another as well as with the control tissues that certain behavior patterns were recognized.

In the majority of transplants that had resided in anterior chambers for more than a week, parts of the original tissue had either remained static, with no apparent further differentiation, or had been overgrown by primitive connective tissue. Interspersed throughout the mesoderm were bronchi in varying stages of development. The quantitative relationships between bronchi and mesoderm usually varied according to the gestational age of the fetus from which the transplant was derived, the length of time that had elapsed since the transplantation and the extent to which the particular portion of the transplant being examined had secured an adequate blood supply from adjacent host tissue. Bronchi examined by means of serial sections were found to be blind tubes or cysts (fig. 20). Some were static, and others showed evidence of terminal branching and growth similar to that observed in fetal lung tissue developing in situ. Studies of serial sections stained for glycogen indicated that terminal growth and differentiation of bronchi in heterotopic lung tissue represented first a chemical and later a structural differentiation of the mesoderm occurring in a manner similar to that observed in utero.

The development of most of some and parts of many of the transplants went on to the formation of the alveolar systems associated with terminal bronchi. Occasionally such differentiation was so balanced and complete that it was impossible to tell from a single field examined under high magnification whether the differentiation of the tissue being examined had occurred in an intact fetus or in an ocular transplant

of fetal lung. Studies of alveolar differentiation by means of serial sections stained for glycogen revealed that in transplants the process was similar to, or identical with, that which occurs in the intact fetus. The

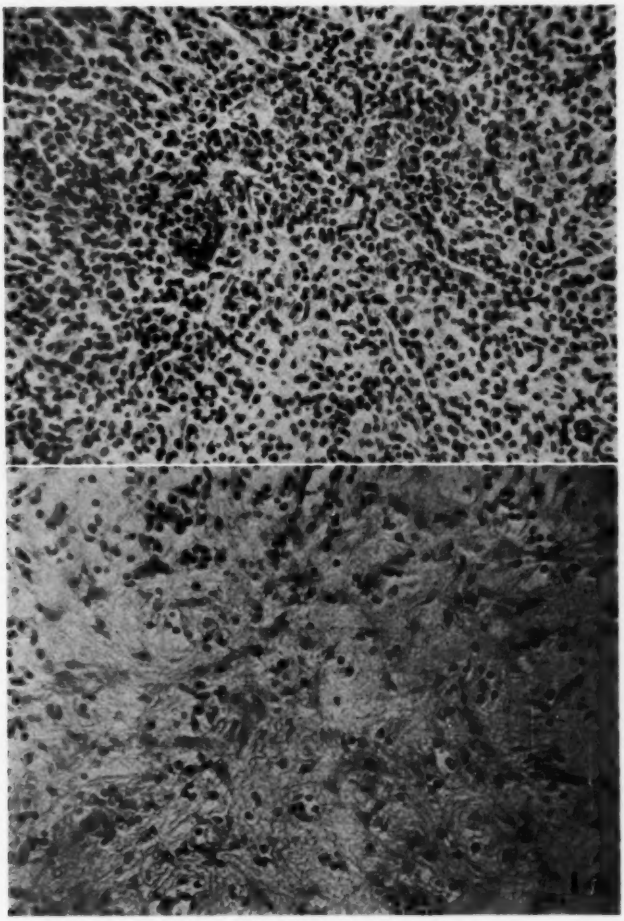


Fig. 13.—Twenty-five day old transplant of fetal guinea pig lung. This tissue was transferred from a 40 mm. embryo to the anterior chamber of an adult guinea pig's eye. Most of the transplant is made up of undifferentiated mesoderm in which there are only occasional abortive attempts at bronchial formation. Hematoxylin and eosin; × 225.

Fig. 14.—Thirty-two day old transplant of fetal guinea pig lung. This tissue was transplanted from a 40 mm. embryo to the anterior chamber of an eye of an adult guinea pig. The relatively acellular, edematous fibrous tissue is near the end stage of the regressive changes seen in figure 12. Hematoxylin and eosin; × 225.

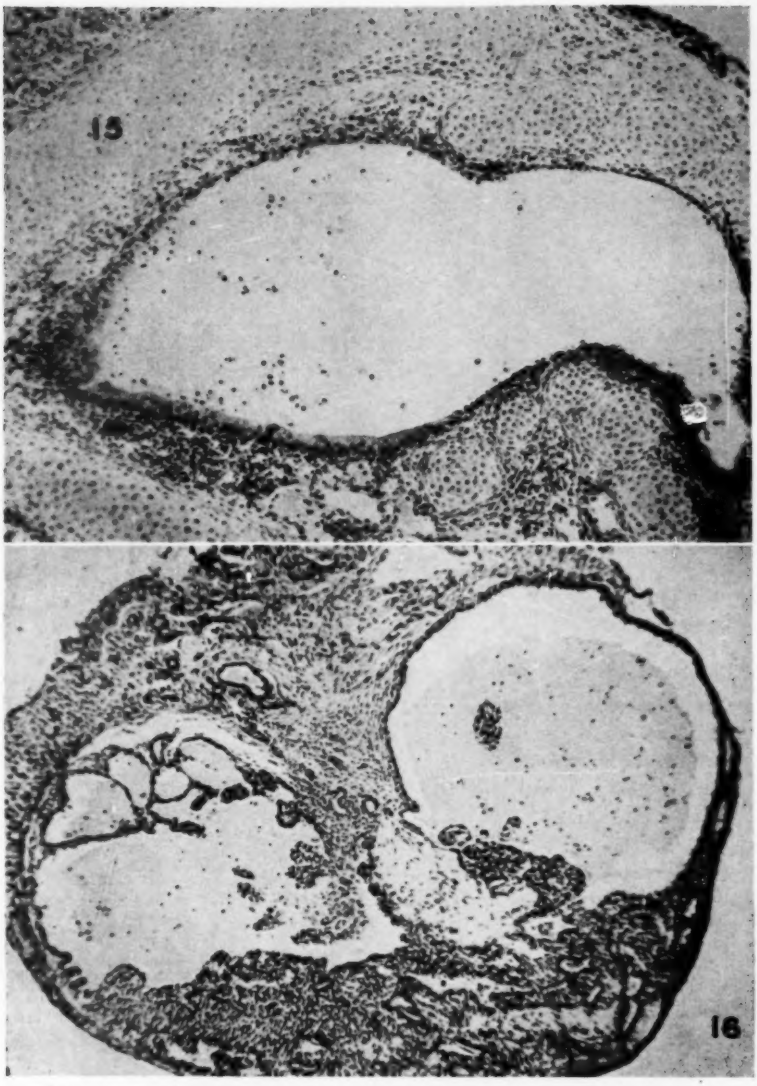


Fig. 15.—A greatly dilated bronchus developing in an 8 day old subcutaneous transplant of fetal mouse lung. This tissue was transplanted from a 14 mm. mouse embryo to an adult mouse. The cartilage is well differentiated. No recognizable smooth muscle is present. The epithelial lining is made up of ciliated columnar epithelial cells in one area but gradually gives way to cuboidal and squamous epithelial cells in other areas about the bronchus (see figs. 17, 18 and 19). Hematoxylin and eosin; × 100.

Fig. 16.—Cysts developing in a subcutaneous transplant of fetal mouse lung. This tissue was transferred from a 15 mm. embryo to an adult mouse and was removed after ten days. Abortive alveolar formation can be seen on the left. These cysts are lined by squamous epithelium which in some areas blends imperceptibly with the surrounding fibrous stroma. Hematoxylin and eosin; × 65.

assertion that in utero the expansion of pulmonary alveoli is related to the patency of bronchial commissures and the aspiration of amniotic fluid was contradicted by the fact that expanded alveoli containing only occasional flattened lining cells were observed in ocular transplants (figs. 8 and 11). Similarly the dilated and even cystic bronchi observed in transplants (figs. 15, 16 and 20) indicated that such alterations are not necessarily predisposed to by extrapulmonary stresses.

The type of bronchial epithelium encountered in the transplants was exceedingly variable. Thus in a single cyst ciliated epithelium was seen to merge gradually into a cuboidal or a stratified layer (figs. 15, 17, 18 and 19). In some instances the variation of the bronchial epithelium appeared to be related to the manner in which the transplant had secured its new blood supply. Normal differentiation tended to occur where an adequate blood supply was obtained soon after transplantation, and abnormal or atypical differentiation was most pronounced where vascularization was poorest. Thus, the cells lining bronchi located in the well vascularized peripheral zone of a transplant usually differentiated in a normal manner, while in areas farther removed from the periphery the epithelium was more commonly of the squamous type. These differences appeared to be due to altered differentiation rather than to metaplasia.

DIFFERENTIATION OF FETAL LUNG TISSUE IN SUBCUTANEOUS TRANSPLANTS

The subcutaneous transplantation site was explored briefly and found to differ in no essential way from the intraocular site as regards ease of growth and differentiation of fetal tissue. The use of mice facilitates the use of larger numbers of animals. The growth of the transplant cannot be visualized, of course. Cyst formation, bronchiectasis and bizarre epithelial overgrowths occurred somewhat more frequently in the subcutaneous transplants than in the intraocular group. Subcutaneous transplants evoked little or no fibrous reaction in mice, whereas an overwhelming proliferation of fibrous tissue occurred about such transplants in guinea pigs. The subcutaneous transplants never attained that degree of differentiation seen in comparable intraocular transplants.

COMMENT

The implications of the foregoing observations are many. They tend to unify and offer a rational explanation for several divergent views concerning pulmonary embryology and pathology, which in the past have occasioned considerable speculation and wide difference of opinion.

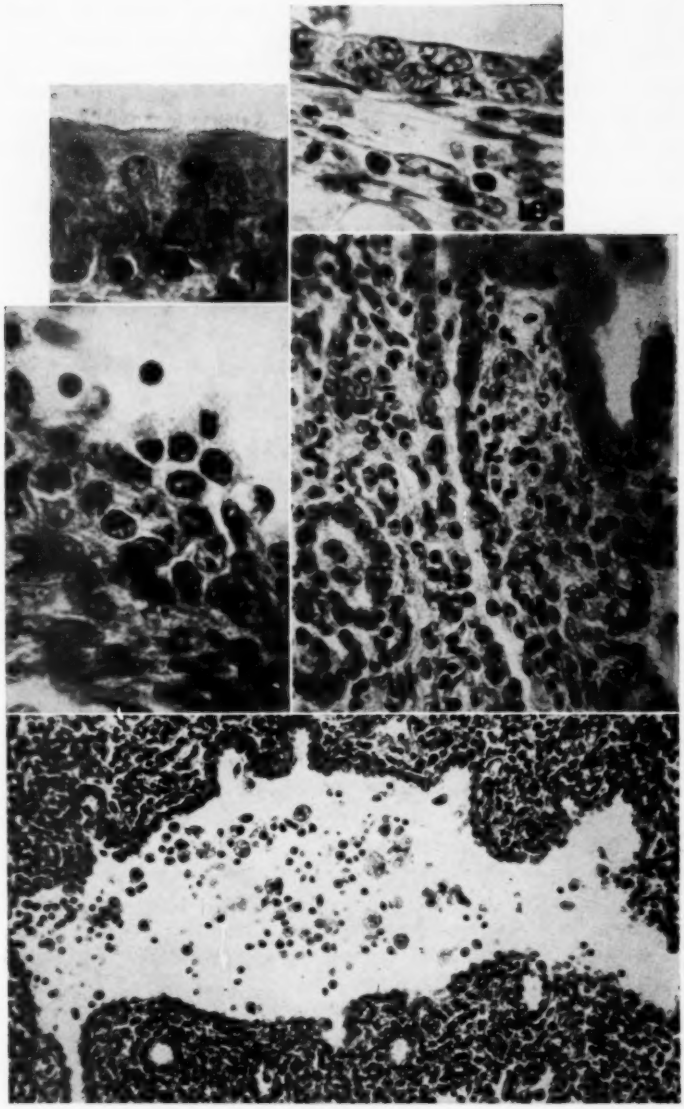


Fig. 17.—Normally differentiated ciliated columnar epithelium lining a portion of the bronchus shown in figure 15. Hematoxylin and eosin; × 900.

Fig. 18.—Stratified cuboidal epithelium lining a portion of the bronchus shown in figure 15. Hematoxylin and eosin; × 900.

The concept of the specificity of germ layers is so firmly implanted in the medical literature of the past one hundred years that it is probably a fact that many have never had occasion to doubt its validity. There is, however, a considerable body of evidence contradicting the doctrine of absolute specificity of the germ layers as enunciated in the last century. Oppenheimer [17] reviewed the subject in 1940 and cited many notable exceptions. In conclusion she stressed such factors as the special metabolism of certain tissues of the developing embryo, the topographic position of the cells and the interactions of various cells, one with another, as being of vital importance in controlling their differentiation.

The results of this study detract from the concept that the ability of a cell to differentiate is necessarily dependent on the germ layer from which it was derived. It would appear that the entire lung is derived from totipotent tissue whose direction of growth and differentiation is induced by the stimulus rather than by the proliferation and migration of previously formed structures.

However radical this concept of bronchial epithelium derived by differentiation from mesoderm may seem there is evidence from other sources to support it. Hunt [18] has shown that in the chick after removal of the presumptive endoderm the mesoderm can form gut and its derivatives. Papanicolaou [18] observed the differentiation by which undifferentiated mesodermal cells became uterine epithelium in the tunica propria of the guinea pig uterus. The prevalent but erroneous idea that mesoderm does not give rise to epithelial structures need cause no difficulty, for there are the uterus and kidney to illustrate this phenomenon.

17. Oppenheimer, J. M.: Quart. Rev. Biol. **15**:1, 1940.

Fig. 19.—Stratified squamous epithelium lining a portion of the bronchus shown in figure 15. Hematoxylin and eosin; × 900.

Fig. 20.—A dilated bronchus in tissue transplanted eight days earlier from a lung of a 44 mm. guinea pig embryo to the anterior chamber of an adult guinea pig's eye. Note that the well differentiated epithelium seen in some areas is continuous in other areas with epithelium which has not yet differentiated from the surrounding mesoderm. Along the lower cyst wall one may observe the manner in which the formation of a bronchus can precede the differentiation by which adjacent mesoderm becomes epithelium; an outpocketing has produced a tube which is as yet nonepithelized. Hematoxylin and eosin; × 225.

Fig. 21.—Longitudinal section of a bronchus developing in a 6 day old subcutaneous transplant of fetal mouse lung. This tissue was moved from a 14 mm. embryo to an adult mouse. Note that the epithelium is continuous with that of the larger bronchus above and that toward the tip of the bronchus it gradually fuses with the surrounding mesoderm. The gradual alteration by which undifferentiated mesodermal cells become epithelium can be seen along the bronchial wall. Hematoxylin and eosin; × 425.

From the point of view of the pathologist the observation of the mesodermal origin of the lung serves to unify many observations which have been incompletely understood. A common genetic origin of the many types of pulmonary neoplasms has been postulated by several observers. The origin of most true pulmonary cancers has been traced to the bronchial epithelium on purely morphologic evidence. However, the marked pleomorphism and the frequent occurrence of sarcomatous elements have been difficult to explain if one considers the bronchial epithelium to be of endodermal origin and this germ layer specific for epithelial structures. The fact that bronchial epithelium can and regularly does differentiate from mesoderm coincides remarkably with its derivatives which appear in neoplasms of the lung.

The difficulty of morphologic classification of cancers of the lung is readily explained and simplified by the consideration of the mesodermal origin of the structures from which they arise. The potencies of bronchial epithelium are those of mesoderm. The sarcomatous changes observed in transplanted pulmonary carcinomas by Stewart, Grady and Andervont [20] and others [21] become more readily explainable. Although these authors draw no definite conclusions from their observations, they were forced to consider the possibility that pulmonary carcinomas were derived at least in part from mesoderm.

Again when one considers the noncancerous bronchial tumors the frequent observation of neoplastic cartilaginous and osseous tissue is not surprising. The necessity of classifying them as mixed tumors as Womack and Graham [22] have done would be abolished.

Finally the bronchiectasis observed in many transplants may have a bearing on the etiology of this disease, especially in those cases in which it is associated with other developmental anomalies. For example, in transposition of the viscera it is possible that the failure of the pulmonary veins to establish connection with the heart and the consequent necessity of persistence of the bronchial veins produce a temporary nutritional defect somewhat similar to that in transplants.

The relatively large cysts that occasionally occur in the transplants, almost replacing them, would seem to be closely analogous to congenital cysts of the human lung.

---

18. Hunt, T. E.: Anat. Rec. **68**:349, 1937.

19. Papanicolaou, G. N.: Am. J. Obst. & Gynec. **25**:30, 1933.

20. Stewart, H. L.; Grady, H. G., and Andervont, H. B.: J. Nat. Cancer Inst. **7**:207, 1947.

21. Wells, H. G.; Slye, M., and Holmes, N. F.: Cancer Research **1**:259, 1941. Breedis, C.; Robertson, T.; Osenkop, R. S., and Furth, J.: ibid. **2**:116, 1942.

22. Womack, N. A., and Graham, E. A.: Arch. Path. **26**:165, 1938.

SUMMARY

The genesis of mammalian pulmonary tissue as observed in the fetus in utero and in transplants in anterior ocular chambers and subcutaneous sites in homologous adults is described. Evidence that the cells which line the bronchi and the alveoli originate from mesoderm is presented, and the nature of the lining of fetal and adult pulmonary alveoli is described. The intracytoplasmic accumulation of glycogen observed in the developing lung has been correlated with the differentiation of bronchi and alveoli. Differences induced in organization and in cytologic differentiation by altering the environment and sources of nutrition of fetal lung tissue are described. Attention is called to the similarity between certain changes induced in fetal lung tissue by transplantation and those encountered in adult lung tissue presumably as a result of congenital dysplasia.

LESIONS INDUCED IN RABBITS BY CHOLESTEROL FEEDING, WITH SPECIAL REFERENCE TO THEIR ORIGIN

ALBERT KUNTZ, Ph.D., M.D.
AND
NORMAN M. SULKIN, Ph.D.
ST. LOUIS

THAT atherosclerosis and xanthomatosis occur in rabbits fed a high cholesterol diet has been reported by many investigators. The earlier literature bearing on experimental atherosclerosis has been reviewed by Anitschkow.[1] The results of studies bearing on certain aspects of the problem have been summarized by various authors, including Weinhouse and Hirsch[2] and Leary.[3] The literature related to xanthomatosis has been summarized particularly by Thannhauser[4] and by Galloway, Broders and Gharmley.[5]

The most prominent cellular components of both the atherosclerotic plaque and xanthoma in the early phases of their development are the so-called foam cells. Nearly all the investigators who have studied these cells have reported data supporting the theory that they are derived from the reticuloendothelial system. According to the point of view advanced by Leary[3] in 1941, the foam cells of the atherosclerotic lesions represent lipophages which invade the intima from the blood stream. Gordon[6] has attempted to explain such invasion on the basis of certain data regarding the behavior of the cellular elements of the circulating blood and theoretic considerations. Contrary to this point of view, Moreton[7] has pointed out that when lipids are present in the blood the nutrient lymph which enters the intima from the blood

---

From the Department of Anatomy, St. Louis University School of Medicine.

This work was supported by a grant from the Life Insurance Medical Research Fund.

1. Anitschkow, N., in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, p. 271.

2. Weinhouse, S., and Hirsch, E. F.: Arch. Path. **30**:856, 1940.

3. Leary, T.: Arch. Path. **32**:507, 1941; **37**:16, 1944.

4. Thannhauser, S. J.: Lipidosis: Diseases of the Cellular Lipid Metabolism, New York, Oxford University Press, 1940.

5. Galloway, J. D. B.; Broders, A. C., and Gharmley, R. K.: Arch. Surg. **40**:485, 1940.

6. Gordon, I.: Arch. Path. **44**:247, 1947.

7. Moreton, J. R.: Science **107**:371, 1948.

plasma carries large lipid particles, which become lodged there. These lipid particles, according to his point of view, incite a local foreign body reaction which results in their being ingested, with formation of typical foam cells. This he regards as the characteristic histologic feature of the genesis and the development of atherosclerotic lesions.

The present investigation was undertaken to obtain additional data relative to atherosclerosis and xanthomatosis in rabbits fed a high cholesterol diet. Particular attention has been given to the cholesterol content of the blood, the origin of the foam cells and the sequence of changes in atherosclerotic lesions following their initiation in both large and small arteries and in the associated lesions of viscera and of the autonomic ganglions and nerves. An attempt has been made also to correlate the histopathologic changes of the autonomic ganglions and nerves with the atherosclerotic and other visceral lesions which are associated with the induced hypercholesteremia.

METHODS

The rabbits used were young adults when the experimental feeding was initiated. The basic diet consisted of a commercial rabbit chow.[8] Twenty animals received, in addition to the basic diet, 1 Gm. of cholesterol in 2.5 Gm. of dehydrogenated vegetable oil daily. Several were fed less than 1 Gm. of cholesterol daily. The diet was prepared by dissolving the cholesterol in melted dehydrogenated vegetable oil and mixing this with chow pellets. The oil, including the cholesterol, became adherent to the chow pellets. In general, all the food given to the individual animal was eaten.

Several of the animals fed the high cholesterol diet were killed 30 to 40 days after the initiation of the diet. Others were killed at graded intervals up to 380 days. Before an animal was killed, its physical condition was noted, and an examination was made to determine the presence or the absence of recognizable xanthoma. In some instances blood pressures were determined. Immediately after the death of the animal, blood was drawn directly from the heart for the determination of its cholesterol content. The tissues desired for study were removed, fixed and prepared by various histologic technics.

BLOOD CHOLESTEROL LEVEL

The rabbits fed the high cholesterol diet became hypercholesteremic relatively early. Determinations carried out on one animal which had received 1 Gm. of cholesterol daily for 46 days and another which had received the same amount daily for 62 days indicated a blood cholesterol level of approximately 3,550 mg.

8. "Purina rabbit chow checkers" (complete ration) was used, made by the Ralston Purina Company, St. Louis. The company has described the chow as follows: The ingredients are wheat germ, soybean oil meal, corn germ meal, alfalfa leaf meal, wheat middlings, ground oats, cornmeal, blackstrap molasses, calcium carbonate and iodized salt. Chemical analysis shows protein 16.45, fat 4.06, fiber 7.93, ash 3.75 and nitrogen-free extract 52.60 per cent. Mineral analysis shows calcium 0.821, phosphorus 0.36, potassium 0.753, sodium 0.317, magnesium 0.150, sulfur 0.222 and chlorine 0.386 per cent.

per hundred cubic centimeters in both animals. Determinations carried out on control rabbits indicated a range in the blood cholesterol level of 137 to 243 mg. per hundred cubic centimeters. Rabbits which were fed 0.5 Gm. of cholesterol or less daily exhibited relatively small elevations of the blood cholesterol levels. One animal fed 0.2 Gm. daily for 200 days showed a level of 1,320 mg. per hundred cubic centimeters. Another fed 0.4 Gm. daily for 87 days showed a level of 1,770 mg. Determinations carried out on rabbits which had been fed 1 Gm. of cholesterol daily for 293 to 380 days indicated a range in the blood cholesterol levels of 2,400 to 2,750 mg. per hundred cubic centimeters.

ATHEROSCLEROSIS

Nature and Distribution of Lesions.—The earliest lesions have been observed in the thoracic aorta. Our data indicate considerable individual variation with respect to the development of atherosclerotic lesions in rabbits fed the same diet. The feeding of less than 1 Gm. of cholesterol daily, furthermore, is less effective than the feeding of 1 Gm. daily. One animal killed 46 days after the initiation of a diet including 1 Gm. of cholesterol daily had early lesions in the aorta and in some other elastic arteries and initial lesions in some of the smaller muscular arteries. The blood cholesterol determination indicated a level in excess of 3,500 mg. per hundred cubic centimeters in this animal. Another rabbit which had been fed 0.4 Gm. of cholesterol daily for 87 days showed only early atherosclerotic lesions, although its blood cholesterol determination indicated a level of 1,770 mg. per hundred cubic centimeters. Rabbits which had been fed 1 Gm. of cholesterol daily for 150 to 200 days had well developed intimal atherosclerotic plaques in the aorta and other large arteries and intimal lesions in many of the small arteries, although most of the smaller arteries, as observed in sections of the viscera, exhibited no recognizable lesions.

In the aorta the maximal thickness of fully developed intimal plaques exceeded twice the thickness of the normal vessel wall. In some instances the plaque extended entirely around the lumen but was not of uniform thickness. Animals having large intimal plaques in the aorta also had large intimal plaques in the common carotid, the coronary and other large arteries, which in most sections did not extend entirely around the lumen. Some relatively small branches of the coronary arteries were partially occluded by intimal thickenings. In other viscera some arteries, including prearteriolar branches, exhibited initial atherosclerotic lesions, but most of the muscular arteries, as observed in sections of the organs, revealed no apparent lesions.

In a rabbit fed the high cholesterol diet 293 days, the blood cholesterol determination of which indicated a level of 2,400 mg. per hundred cubic centimeters, the intimal plaques in the aorta were no larger than those observed in the aortas of rabbits which had been fed cholesterol for only 200 days, but they differed from the latter in their composition. The most striking differences were a marked increase in the amount of the collagenous tissue and a marked decrease in the number of foam cells present. Most of the remaining foam cells exhibited shrinkage of both the cytoplasm and the nucleus. Sections of the aorta of this rabbit revealed extensive lesions of the media, particularly in areas in which the intimal plaques were most extensively developed. In some areas destruction of the media was almost complete (fig. 2).

Sections of the common carotid arteries of this animal exhibited intimal plaques comparable in size to those observed in the carotid arteries of rabbits fed cholesterol only 200 days. These plaques also showed a proportionate increase of the amounts

of collagenous tissue present (fig. 1). Sections of the coronary arteries exhibited lesions which were comparable to those in the common carotid arteries.

The percentage of the arteries in the viscera which presented atherosclerotic lesions, as observed in sections of the organs, was markedly greater than that noted in the animals which had been fed cholesterol only 200 days or less. Occasional small arteries observed in sections of various organs, including the heart, the intestine and the kidney, were completely or almost completely occluded. Most of the very small arteries and the arterioles exhibited apparent thickening of the walls and constriction in some degree.

In rabbits which had been fed cholesterol for 300 to 380 days the atherosclerotic lesions were not appreciably more extensive than in the rabbits fed cholesterol for approximately 300 days. The damage of the media of the arteries was somewhat more extensive. The proportionate amounts of collagenous material in the lesions also was greater. In many instances sections revealed definite stratification due to alternating layers characterized respectively by preponderance of fibrous tissue and foam cells. Some of these lesions showed, in contact with the inner elastic membrane, a relatively thick layer, which consisted almost exclusively of foam cells and intercellular lipid material (fig. 2).

Initial atherosclerotic plaques consisted mainly of foam cells and intercellular lipids located between the endothelium and the internal elastic membrane (fig. 3). Fibrous tissue including cellular elements of connective tissue origin became apparent early and were constantly present in the later lesions.

Sections of the aortas of cholesterol-fed rabbits stained with sudan black exhibited lipid material in larger amounts. In the atherosclerotic lesions lipids were present both within the cells and between them. The intracellular lipids appeared mainly in the forms of granules and globules of varying sizes. In early lesions the foam cells were heavily laden with lipids. In the more advanced lesions most of the foam cells contained but little stainable lipid material. In the media the lipids appeared mainly in granules and globules of varying sizes lying between the elastic fibers. Sections of the aortas of control rabbits, stained in the same manner, contained only traces of lipids except in the adventitia.

Sections of arteries of cholesterol-fed rabbits stained with nile blue sulfate, which is specific for unsaturated fatty acids, exhibited these substances in large amounts, whereas sections of the same arteries of control rabbits, stained in the same manner, exhibited only traces of unsaturated fatty acids. In general, the distribution of the unsaturated fatty acids paralleled that of the lipids which reacted positively to sudan black. The material which reacted positively to the nile blue sulfate probably represents triolein derived from the dehydrogenated vegetable oil in which the cholesterol had been dissolved in the preparation of the diet.

Sections of arteries of cholesterol-fed rabbits subjected to the Romieu test [8a] for cholesterol exhibited large amounts of this material in the atherosclerotic plaques, both in the foam cells and in the ground substance, but none in the media or in the adventitia.

Development of Lesions.—The initial atherosclerotic lesion appears as a slight thickening of the intima. The area of the lesion may include a few foam cells located between the endothelium and the inner elastic membrane. In some instances the endothelial cells in the area of the lesion appear to be thickened and to contain lipid material in their cytoplasm. They are also more numerous than in unaltered areas. This suggests endothelial cell proliferation. In some instances there were observed just beneath the endothelium, cells which, with respect to size, form and

8a. Romieu M.: Compt. rend. Acad. d. sc. **184**:1206, 1927.

lipid content, appeared to represent intermediate stages of a transformation of endothelial cells into foam cells. During their early phases many of the plaques consist essentially of aggregates of foam cells in the intima, which are covered with endothelium. An individual lesion usually involves a circumscribed area the greatest dimension of which is parallel to the axis of the vessel. It is narrow at first but may increase in width until it extends completely around the lumen of the vessel. The tumor, which has been designated the intimal plaque, is of variable thickness. As it increases in size, fibrous tissue becomes apparent within it. The earliest connective tissue cells and fibers appear between groups of foam cells. As the fibers become more abundant, the groups of foam cells become more widely separated. In the denser parts of the fibrous tissue foam cells in small groups or individually appear to lie embedded in a fibrous matrix. These cells are smaller than the typical foam cells, and their nuclei are shrunken. They have lost part of their lipid content. In the larger masses, particularly in those close to the endothelium, the foam cells remain unaltered. The fibrous material in the plaque, as determined by differential staining methods, is mainly collagen. Fibroblasts appear mainly between the collagenous fibers. In rabbits which had been fed cholesterol 300 days or longer, many of the intimal plaques consisted mainly of connective tissue, with relatively small masses of foam cells embedded in it.

In the area in which the plaque is thickest, in most instances some tissue elements of the intima and the inner layers of the media appear necrotic. As collagenous fibers develop in the intimal plaque, such fibers arise also in the portion of the media in which necrosis has taken place. In many instances the media was materially reduced in thickness in the area covered by an intimal plaque, owing to destruction of both muscular and elastic elements. In some instances the media was almost completely destroyed in a limited area (fig. 2). Not infrequently, small groups of foam cells were observed in the media in areas in which the muscular and the elastic elements had undergone necrosis. Calcium deposits occurred in greatest abundance in these areas. In certain instances groups of foam cells appeared also in the adventitia overlying the lesion of the media. Lesions of this kind which involve the entire thickness of the arterial wall undoubtedly constitute areas of profound weakness.

The atherosclerotic plaques of the small muscular arteries, as observed in sections of viscera, in most instances did not extend entirely around the lumen but consisted of one or more groups of foam cells and some collagenous fibers (fig. 4). In many sections the endothelium appeared to be intact. Even a minute lesion causes partial occlusion of the lumen. In many instances occlusion of the lumen was complete. Reduced thickness of the media was not uncommon.

In the smallest arteries complete occlusion was not uncommon even though the lesions were not extensive and had not yet reached the fibrotic stage. In an arteriole a group of no more than 3 or 4 foam cells may cause complete occlusion.

Genesis of Foam Cells.—Our observations relative to the origin of the so-called foam cells which make up the major portion of the intimal plaque in the early phases of its evolution support the theory that they arise locally and are derived mainly from the endothelium. In sections of the aorta and other large arteries which exhibit early lesions the endothelium in the area of the lesion, in many instances, was thicker than the unmodified endothelium, and the endothelial nuclei were closer together. The endothelial cells apparently had increased in number, suggesting endothelial cell proliferation. Some of the thickened endothelial cells were so laden with lipid material that their cytoplasm presented a vacuolated appearance resembling that of the cytoplasm of the foam cells beneath the endo-

thelium. Mitotic division of endothelial cells has not been observed and probably does not occur in the developing atherosclerotic lesion. In some thickened endothelial cells the nucleus appeared to be constricted in a plane at right angles to its long axis. Binucleate endothelial cells also have been observed. Dumbbell figures

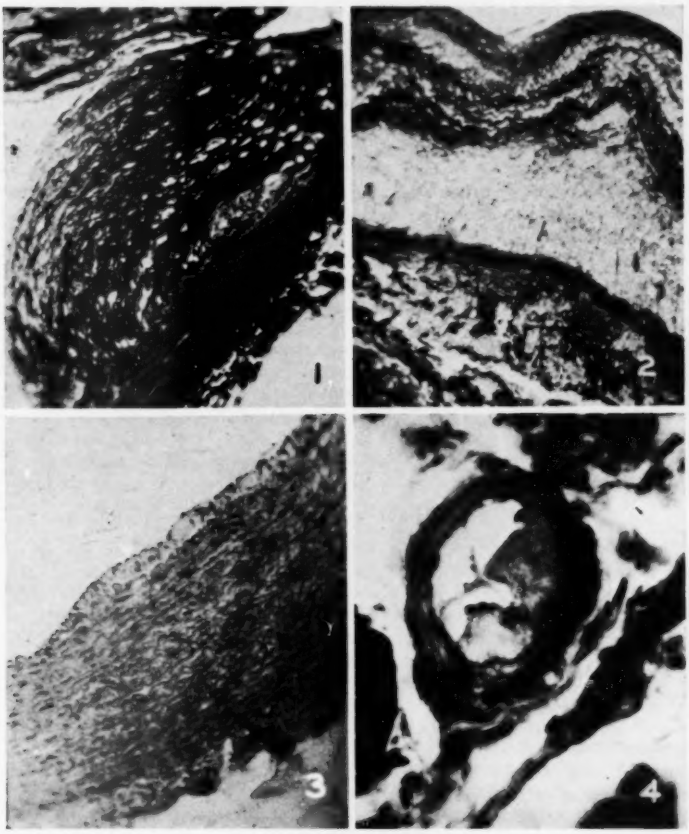


Fig. 1.—Section of an atheromatous carotid artery showing an intimal plaque.
Fig. 2.—Section of an atheromatous aorta with stratification of an atheroma and partial destruction of the media.
Fig. 3.—Section of an aorta showing an early atheromatous lesion.
Fig. 4.—Section of a small atheromatous artery of the wall of the intestine, partially occluded by foam cells.

such as are characteristic of nuclei undergoing amitotic division have not been observed in the endothelium. Such figures have been observed frequently just beneath the endothelium and in the deeper portions of intimal plaques. In intimal plaques which were undergoing rapid development, cells which appeared to represent

intermediate stages, with respect to form and lipid content, between the thickened endothelial cells and the fully differentiated foam cells were not uncommon just beneath the endothelium.

Thickening of endothelial cells and assumption of spheroid forms as the cytoplasm becomes laden with lipid material have been observed frequently in small arteries. Such cells protrude into the lumen, sometimes singly and sometimes in small groups. In sections of the smallest arteries the foam cells retained their positions in the endothelium. In sections of arteries which were large enough to permit the development of intimal plaques comprising many foam cells, the latter usually appeared to be covered by endothelium.

Early atherosclerotic lesions appear to be limited to the intima. If all the foam cells arise locally, those in the lesions of the smaller arteries must be derived solely from the endothelium, for in these vessels the intima includes no other cellular elements. In the larger arteries the intima includes some cells of connective tissue origin, some of which undoubtedly have the capacity to become laden with lipids and to differentiate into foam cells. Even in these vessels the endothelium apparently constitutes the chief source of the foam cells.

In order to test the theory that the foam cells in atherosclerotic lesions are derived from phagocytic cells which are transported in the circulating blood, some rabbits with marked hypercholesteremia and early atherosclerotic lesions were subjected to intravenous injection of dilute india ink at two day intervals for three successive injections. Others were given intravenous injections of a neutral suspension of lithium carmine. In preparations of the tissues of the animals which had been given injections of india ink the phagocytic cells were laden with carbon granules but no carbon granules could be observed in the foam cells of the atherosclerotic lesions. In preparations of the tissues of the animals which had been given injections of lithium carmine abundant carmine granules were present in the cytoplasm of the phagocytic cells but none were observed in the foam cells of the atherosclerotic lesions.

In sections of the viscera of the animals given injections of india ink or of carmine, particularly in those of the spleen and the liver, carbon granules and carmine granules, respectively, were observed in many foam cells. These cells obviously represent phagocytic cells which have taken up lipid material and have become foam cells. Foam cells containing carbon or carmine granules have not been observed in the arterial lumens. Smears of the blood of rabbits which had been given injections of india ink or of lithium carmine, likewise, exhibited no cells which had taken up the injected substances. Free foam cells were observed in the lumen of an artery—the aorta—in only one of our animals. This animal had received injections of lithium carmine, but the cells in question had taken up none of the material. They probably represented foam cells which had become detached from an intimal plaque of the aorta. Our observations lend no support to the theory that phagocytic cells transported in the circulating blood constitute a source of the foam cells observed in atherosclerotic lesions.

Genesis of Collagenous Tissue in Atherosclerotic Lesions.—Initial atherosclerotic lesions include no fibrous elements. Lesions of large arteries include some large collagenous fibers and some cells of connective tissue origin relatively early. The source of the cells in question undoubtedly is the meager connective tissue component of the intima. With development of the lesion the connective tissue elements increase in numbers. In rapidly growing intimal plaques nuclei of fibroblasts have been observed in amitotic division. Mitotic division of the nuclei of fibroblasts has not been observed. The collagenous nature of the fibrous elements has been determined by differential staining.

In instances in which the inner elastic membrane had broken down and the atherosclerotic lesion had extended into the media, collagenous fibers could be traced from the media into the intimal plaque. In sections through such lesions an increase in the number of fibroblasts in the media also was apparent.

XANTHOMATOSIS

All the rabbits which had been fed a high cholesterol diet long enough for extensive atherosclerosis to have developed had xanthomatosis also. The tumors were associated mainly with synovial membranes and tendon sheaths. In all the tumors associated with the larger joints which have been carefully examined a definite relationship to the joint cavity could be demonstrated. In some of the animals xanthomatosis was widespread. Demonstrable tumors were present in relation to nearly all the joints of the extremities, the intervertebral articulations and the temporomandibular joints. It was not uncommon for tumors to extend along tendon sheaths. The tumors varied in size within a wide range. Many of the smaller ones were hardly large enough for gross demonstration. Some of the larger ones showed maximum dimensions of more than 5 cm. In general they were firm and tough, but when cut through they were found to include soft areas, from which a whitish, creamy substance could be expressed with slight pressure. Some were soft and contained small amounts of whitish fluid.

The histologic structure of these tumors resembled that of the intimal plaques of the atherosclerotic arteries (fig. 5). They were composed mainly of masses of cells which were identical in appearance with the foam cells of the atherosclerotic lesions. A large lipid content of the tumors was demonstrated also by the use of fat stains. Some fibrous elements were constantly present between the masses of foam cells. In the more advanced tumors the fibrous tissue was more prominent than in the earlier ones. This fibrous tissue, like that in the arterial lesions, comprised mainly collagenous elements, as was demonstrated by differential staining. With the appearance of collagenous fibers, fibroblasts also became apparent in the tumor. As the collagenous tissue increased in volume, the groups of foam cells became more widely separated. Many of the foam cells also underwent reduction in size.

Our observations relative to the genesis of the foam cells in xanthoma are less complete than those relative to the genesis of the foam cells in the atherosclerotic lesion. Since the xanthoma cells appear to be identical in histologic structure with the foam cells of the atherosclerotic lesion, they probably have a similar origin. The earliest foam cells of xanthoma are located just beneath the mesenchymal epithelium of the synovial membrane. The foam cells of the early stage of xanthoma undoubtedly arise mainly from mesenchymal epithelial cells and probably histiocytes located in the synovial membrane. As the tumor becomes vascular, additional foam cells may be derived from the vascular endothelium. Amitotic division of young xanthoma cells also has been observed.

ASSOCIATED VISCERAL LESIONS

In all the rabbits which showed advanced atherosclerosis, lesions comprising foam cells were present in the viscera, particularly in the liver, the adrenal glands, the spleen and the kidneys.

In the liver Kupffer cells and other endothelial cells became transformed into foam cells. The hepatic cells also became laden with lipid material to the extent that in ordinary preparations many of them presented the vacuolated appearance

characteristic of foam cells. Lipid material has been demonstrated in these cells also by the use of fat stains. Sections of the livers of some of the animals exhibited areas in which the hepatic cells had undergone necrosis.

The adrenal glands of cholesterol-fed rabbits became markedly enlarged. In some of the animals these glands were more than twice their normal size. The enlargement was due in part to distention of the lymphatic vessels, particularly of those of the medulla, with masses of foam cells and in part to hypertrophy of cortical cells caused by cytoplasmic storage of excessive amounts of lipid material. The loading of the cortical cells with lipid material appears earliest in the fasciculate zone. Later it becomes apparent also in the reticular and glomerular zones. In animals fed cholesterol 200 days or longer the lymphatic vessels of the adrenal medulla were widely distended with foam cells. These cells formed compact masses, some of which gave evidence of necrosis in their central areas. Since the endothelium of the lymphatic channels of the adrenal glands represents a site of active proliferation of reticuloendothelial cells, it may be assumed that the foam cells in these channels were derived from the endothelium.

As the lymphatic vessels of the adrenal medulla became packed with foam cells, many of the chromaffin cells disappeared. In the glands in which the greatest distention of the lymphatic vessels was observed, few chomaffin cells could be detected. Those which remained reacted only lightly to the basic stains. The reduction of the volume of chromaffin tissue undoubtedly results in a decrease of the output of adrenin.

In sections of the spleens of rabbits with atherosclerotic lesions, foam cells were present particularly in the venous sinuses of the red pulp. In most of the cholesterol-fed animals the red pulp was congested and much increased in volume. The white pulp occupied a relatively small percentage of the area of the section. Its appearance suggested an actual reduction of its volume, although the spleen was appreciably enlarged. Many of the cells which had become differentiated into foam cells in the red pulp had ingested fragments of red blood cells and other particulate matter, indicating their capacity for phagocytosis. Foam cell differentiation of large lymphocytes also could be observed. The foam cells of the spleen exhibited wider variations in size than those of the liver or those of the adrenal glands. They were not closely packed in the venous sinuses, although they were present in large numbers.

Sections of the kidneys of all the rabbits with atherosclerotic lesions induced by cholesterol feeding presented involvement of the renal tubules. In the early lesions some of the epithelial cells, particularly those of the limbs of Henle's loops, appeared finely vacuolated, owing to lipid material in the cytoplasm. These cells reacted only lightly to the ordinary stains. Foam cells, either single or in small groups, could be observed between renal tubules. In later phases of cholesterol feeding the renal lesions were larger. Increasingly larger numbers of epithelial cells gave evidence of lipid material in the cytoplasm. In some areas, particularly in the outer zone of the medulla, the epithelium of several adjacent tubules was necrotic, and crystals were apparent in the necrotic mass. Foam cells were present between the renal tubules at many points. In some instances the masses of foam cells were sufficiently large and compact to press adjacent tubules apart. Clumps of cells laden with lipid material appeared also in the lumens of some renal tubules. These cells appeared to be detached epithelial cells. In oblique sections of tubules columns of cells could be observed, in certain instances, extending from the intact epithelium into the lumen. The epithelial cells of some of the proximal convoluted tubules exhibited vacuolation of the cytoplasm. In these cells the accumulation of lipid material was less impressive than in those of the limbs of Henle's loops. No other definite pathologic changes have been observed in the renal cortex.

In the kidneys the foam cells probably represent mainly cells which have been derived locally from the vascular endothelium. The lesions observed in the kidneys were sufficiently extensive to impair renal function to a high degree.

AUTONOMIC GANGLIONS AND NERVES

In all the cholesterol-fed rabbits in which atherosclerosis was well advanced the sympathetic ganglions and nerves exhibited recognizable alterations. Both the ganglion cells and the neuroglial tissue of the ganglions reacted positively to fat

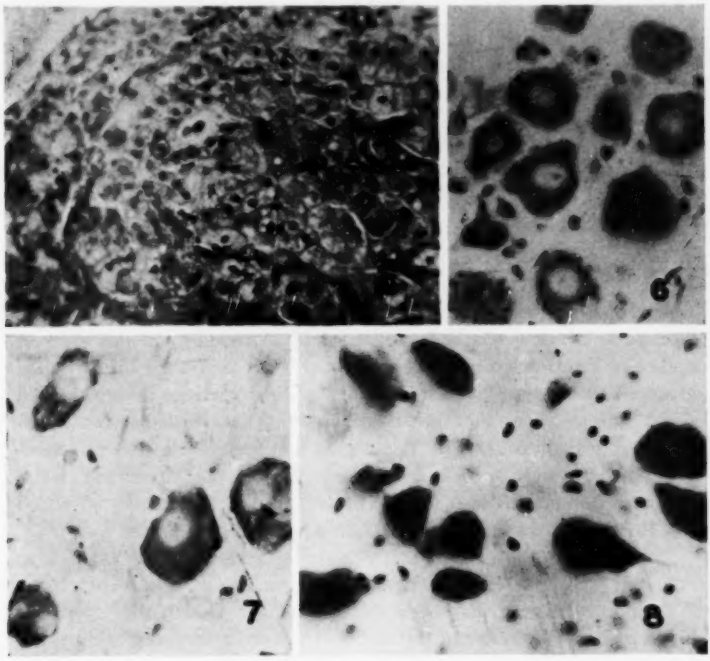


Fig. 5.—Section of a xanthoma.

Fig. 6.—Section of an autonomic ganglion of a control rabbit.

Fig. 7.—Section of an autonomic ganglion of a rabbit which had been fed cholesterol for 80 days.

Fig. 8.—Section of an autonomic ganglion of a rabbit which had been fed cholesterol for 280 days.

stains. Traces of lipid material, exclusive of myelin, were present also in the nerves. In sections which had been treated with fat solvents the cytoplasm of the ganglion cells was finely vacuolated, owing to extraction of its lipid content. Glycogen could not be demonstrated in the ganglion cells by the Bauer technic. The ganglion cells of control animals reacted positively to this technic. The chromidial content of the ganglion cells was diminished. The chromidial sub-

stance left in the cytoplasm was present mainly in the state of chromidial dust or in solution (figs. 6, 7 and 8). The ascorbic acid content of the ganglion cells was undoubtedly diminished. Many of the cells appeared to be entirely devoid of it. In the ganglions of rabbits fed cholesterol 300 days or longer, most of the ganglion cells were shrunken to some degree and retained but little chromidial substance. They were also devoid of glycogen and almost devoid of ascorbic acid. The succession of alterations observed in the ganglion cells and their appearance in the later phases of cholesterol feeding suggest, not hyperactivity, but functional depression.

COMMENT

The data obtained in this investigation indicate that rabbits fed a high cholesterol diet exhibit profound metabolic disturbances relatively early and that a condition of heterostasis persists throughout the period of cholesterol feeding. Our data relative to the increased amount of lipid material in the blood and the elevation of blood pressure in cholesterol-fed rabbits agree in general with the data reported by other investigators who have studied these aspects of the problem. Our data relative to the initiation of atherosclerotic lesions and their distribution in rabbits agree in general with those of Leary [8] and Wilens.[9] Those relative to the origin of the foam cells support the theory that these cells arise locally mainly from the endothelial lining of the vessels. This point of view has been advanced by Altschul [10] on the basis of studies of arteriosclerotic lesions in man. It is supported also by data advanced by Moreton,[7] which indicate that lipid particles become lodged in the intima where they are taken up by cells, which become transformed into foam cells. The assumption that phagocytic cells of reticuloendothelial origin which are transported in the circulating blood become differentiated into foam cells and invade the intima, advanced by Leary,[8] is incompatible with our findings.

The similarity in cytologic structure of atherosclerotic plaques in man and in cholesterol-fed rabbits, particularly in the early phases of their development, has been emphasized by Anitschkow.[1] Because of the high lipid content of the blood of the cholesterol-fed rabbits, the intimal plaques develop much more rapidly in the vessels of rabbits than in those of man. Late lesions of man, consequently, cannot be compared directly with late lesions of experimental rabbits. The former usually consist almost exclusively of fibrous tissue, owing to the extensive growth of collagenous fibers and the disappearance of the foam cells as the lipid material is resorbed. In some instances, however, the differentiation of foam cells continues in atherosclerotic lesions in man even during the later stages of these lesions.

9. Wilens, S. L.: Am. J. Path. **18**:63, 1942.
10. Altschul, R.: Arch. Path. **38**:305, 1944.

A reduction of the number of foam cells, as well as of the sizes of many of those which remain, concurrent with an increase of the connective tissue components of the itimal plaque, is a prominent feature of atherosclerotic lesions in their later phases in cholesterol-fed rabbits. This is in full accord with the observation of Anitschkow [1] that almost complete fibrous transformation of atherosclerotic lesions may take place in experimental rabbits if the animals are kept alive two to three years after discontinuance of the cholesterol feeding. From the structural point of view, therefore, the atherosclerotic lesions of cholesterol-fed rabbits are essentially similar to the atherosclerotic lesions of man.

In the rabbit xanthomatosis appears to be due to the same causes as atherosclerosis. Our data relative to the initiation and the evolution of xanthoma in cholesterol-fed rabbits agree in general with those of Rusch, Bauman and Kline.[11] This tumor conforms closely in its cytologic structure to xanthoma of the tendon sheaths and the synovial membranes of man as described by Galloway and co-workers.[5] As far as our data have a bearing on the origin of the xanthoma cells, which appear to be identical with the foam cells of the atherosclerotic lesion, they support the assumption that these cells arise locally from the mesenchymal epithelium of the synovial membranes and probably from undifferentiated cells of the adjacent connective tissue.

The visceral tumors including foam cells which developed in cholesterol-fed rabbits also appeared to be due to the same causes as the atherosclerotic lesions. They did not play a primary role in the development of the atherosclerotic lesions. In view of the extent to which they developed in all the animals which had been fed cholesterol 90 days or longer, particularly in the liver, the spleen, the adrenal glands and the kidneys, they undoubtedly resulted in functional impairment of these organs in some degree. The paucity of the chromaffin tissue remaining in the adrenal medulla suggests material reduction of the output of adrenin. The extensive damage of the renal tubules also suggests extensive impairment of renal function. Impairment of renal function undoubtedly is a factor in the development of the hypertension and, consequently, in the atherosclerosis.

Our data relative to the vasomotor nerves do not indicate exaggerated vasomotor activity but indicate rather functional depletion of the vasomotor mechanisms due to faulty metabolism, including that of the sympathetic ganglion cells. If the output of adrenin is materially decreased, as is suggested by the reduction in the amount of chromaffin tissue in the adrenal medulla, this would tend further to reduce sympa-

11. Rusch, H. P.; Bauman, C. A., and Kline, B. E.: Arch. Path. **28**:163, 1939.

thetic tonus. Vasomotor nerve activity, consequently, cannot be regarded as a significant factor in the production and the maintenance of the elevated blood pressure of these animals.

SUMMARY

In rabbits fed cholesterol daily in addition to a rabbit chow hypercholesteremia and hypertension developed. In those fed 1 Gm. of cholesterol daily atherosclerotic lesions appeared in the aorta and other large arteries within 30 days. As the process advanced lesions appeared also in small arteries. The initial atheroma consisted mainly of a few foam cells in the intima. As the number of foam cells increased, a thick intimal plaque was formed. Collagenous fibers soon appeared in the plaque. Associated with these fibers were some fibroblasts. The late lesion included a large percentage of fibrous tissue, including numerous fibroblasts. In sections the late intimal plaque exhibited stratification due to alternate arrangement of the fibrous tissue and the foam cells. Early atherosclerotic lesions were limited to the intima; many of the older lesions extended into the media.

The data support the assumption that the foam cells observed in atherosclerotic lesions are derived locally from the endothelium. The fibrous tissue is derived from connective tissue elements of the intima and the media.

Xanthomatosis occurred in all rabbits with advanced atherosclerosis. The data relative to the origin of the xanthoma cells support the assumption that these also arise locally from the mesenchymal epithelium and from undifferentiated cells of the synovial membranes. In rabbits with advanced atherosclerosis, lesions characterized by foam cells developed also in viscera, particularly in the liver, the spleen, the adrenal glands and the kidneys. There also the foam cells appeared to arise locally. The autonomic ganglions and nerves exhibited a succession of alterations which in the later phases of cholesterol feeding suggested functional depression of the cells.

THE PHENOMENON OF LEUKERGY

LUDWIK FLECK
AND
ZOFIA MURCZYNSKA
LUBLIN, POLAND

LEUKERGY is a phenomenon found in citrated blood (Fleck, 1942) which manifests itself as an agglomeration or clumping of leukocytes. The clumps contain up to 20 or more cells with marked tendency to cellular homogeneity. It appears in infectious diseases in man and animals, and it can be experimentally elicited by an intravenous injection of live or killed gram-negative bacteria (e. g., Bacterium coli, Salmonelli typhi, Bacterium proteus X) or by an intrapleural injection of turpentine. A report of our earlier studies on leukergy has been published.[1] The term "leukergy" is derived from three words of Greek origin: λευκός κύτος (white cell) and ἐργεῖν to act).

An improved technic was used, as follows:

Tube Test.—The sample of blood is drawn either from a vein or from the heart of the animal. It is then mixed with a 3.8 per cent sodium citrate solution in the proportion of 4:1 and incubated. After one, two, or three hours of incubation a drop of the well mixed blood is taken with a large platinum loop and placed on a slide as a large drop. The slide is rocked gently, dried in the incubator and either stained with Wright stain or fixed by heat and then stained with an aqueous solution of methylene blue. The smears may be hemolyzed. This is best done by placing the slides with the dried drops in the ice box for several minutes and subsequently exhaling several times on the cooled slides following their removal from the refrigerator. The condensing steam lakes the blood without washing off the drop. This technic differs from that previously described in that we use whole blood without attempting to concentrate leukocytes by either sedimentation or centrifugation.

Drop Test.—A drop of a 2 per cent sodium citrate solution is thoroughly mixed with an equal drop of blood on a microscopic slide previously coated with a 1 per cent alcoholic solution of brilliant cresyl blue. The slide is then incubated for 15 to 20 minutes in a moist chamber and can be examined microscopically either with a dry lens or with oil immersion after drying of the drop followed by gentle rocking. Hanging drops of citrated blood with or even without brilliant cresyl blue observed on a heated microscopic table give also clearcut pictures of leukergy.

In figure 1, *A* and *B* show a positive and a negative drop test, and *C* a positive tube test, with remarkable homogeneity of the groups.

The results of the tube test are in general more reliable. The drop test is quick and simple.

From the Department of Microbiology, M. Curie-Sklodowska University.

1. Fleck, L., and Murczynska, Z.: Texas Rep. Biol. & Med. **5**:156, 1947.

CYTOLOGIC SELECTIVITY OF LEUKERGY

The phenomenon shows two kinds of cytologic selectivity.

The degree of agglomeration of various kinds of white blood cells is different: i.e., the percentage of clumped leukocytes, lymphocytes or monocytes varies in different cases. In most instances the clumping involves mainly polymorphonuclear leukocytes.

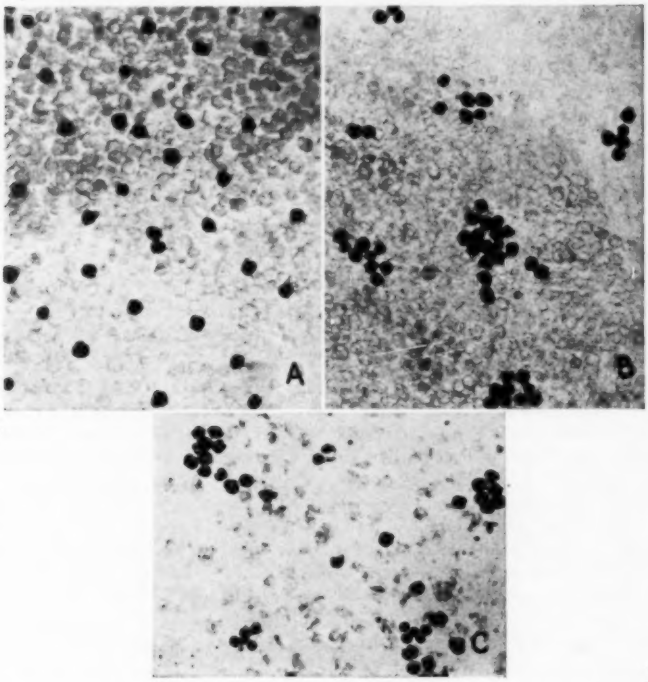


Fig. 1.—*A*, normal blood in drop test; *B*, leukergy in drop test; *C*, tube test of rabbit after intravenous injection of killed colon bacilli.

There is a marked tendency to form cytologically homogeneous groups, i.e., groups containing either neutrophils, or lymphocytes, or monocytes.

Both features of selectivity are well demonstrated in table 1, which gives the counts in a sample from a tube test stained with Wright stain in a case of toxic dermatitis, and in following tables. The inequality of the process, which involves 81 per cent of eosinophils and only 14 per cent of lymphocytes, is obvious. Larger groups (from 5 cells up)

contained 3 per cent lymphocytes and 39 per cent neutrophils, although the respective total numbers in the counted area of the smear were approximately equal. The nine larger groups consisted of 81 cells. They are shown in table 2.

TABLE 1.—*Leukergy in Toxic Dermatitis, Case 1, Sample 1*

	Neutrophils	Eosinophils	Lymphocytes	Monocytes	Total
Counted number of scattered and agglomerated cells	68 (29%)	90 (39%)	65 (28%)	9 (4%)	232
In groups from 3 cells up	49 (72%)	73 (81%)	9 (14%)	2 (22%)	133 (57%)
n groups from 5 cells up	27 (39%)	50 (55%)	2 (3%)	2 (22%)	81 (34%)
Scattered cells	19 (28%)	17 (19%)	56 (80%)	7 (78%)	99 (43%)

TABLE 2.—*Composition of the Nine Larger Groups in Case 1, Sample 1*

	Eosinophils Only	Neutrophils Only	Mixed Groups	Total Groups	Total Cells
Groups of 5 cells	1	1	..	2	10
Groups of 6 cells	..	..	1	1	6
Groups of 7 cells	1	..	1	2	14
Groups of 10 cells	..	..	1	1	10
Groups of 11 cells	..	..	1	1	11
Groups of 13 cells	..	..	1	1	13
Groups of 17 cells	..	..	1	1	17
Total	..	..	..	9	81

TABLE 3.—*Leukergy in Toxic Dermatitis, Case 1, Sample 2*

	Neutrophils	Eosinophils	Lymphocytes	Monocytes	Total
Counted number of scattered and agglomerated cells	72 (35%)	84 (41%)	39 (19%)	11 (5%)	206
In groups from 3 cells up	45 (62%)	73 (87%)	6 (15%)	3 (27%)	127 (62%)
In groups from 5 cells up	29 (40%)	48 (57%)	5 (13%)	..	82 (40%)
Scattered cells	27 (38%)	11 (13%)	33 (85%)	8 (73%)	79 (38%)

TABLE 4.—*Composition of the Ten Larger Groups in Case 1, Sample 2*

	Eosinophils Only	Neutrophils Only	Mixed Groups	Total Groups	Total Cells
Groups of 5 cells	1	1	..	2	10
Groups of 6 cells	..	..	2	2	12
Groups of 7 cells	1	..	2	3	21
Groups of 11 cells	..	..	1	1	11
Groups of 13 cells	..	..	1	1	13
Groups of 15 cells	..	..	1	1	15
Total	..	..	..	10	82

In a second drop of blood taken simultaneously in the same case we found the numbers given in table 3.

The conformity of both results is fairly good, at least in respect to neutrophils and eosinophils. The larger groups (from 5 cells up) in this preparation contained 82 cells. They are shown in table 4.

The total of nineteen groups (9 + 10) from 5 cells upward contained 163 cells:

Eosinophils:	50 + 48 cells	=	98	= 60%
Neutrophils:	27 + 29 cells	=	56	= 34%
Others:	4 + 5 cells	=	9	= 6%
			163	= 100%

All four groups of 5 cells were homogeneous. If the number of cytologically homogeneous groups depended only on the percentage of this special kind of cells available for agglomeration (i.e., if there were no tendency to form homogeneous groups), then the expected frequency of homogeneous 5 cell groups in this case would be:

For eosinophils $\left[\frac{60}{100}\right]^5 = 0.08 = 8\% \pm 13$ and it is 50 per cent.

For neutrophils $\left[\frac{34}{100}\right]^5 = 0.0045 = 0.45\% \pm 3.1$ and it is 50 per cent.[2]

Of five 7 cell groups, 2 were pure eosinophil groups, which makes 40 per cent. The expected number (if one assumes that there is no tendency to form homogeneous groups) would be:

$\left[\frac{60}{100}\right]^7 = 0.028 = 2.8\% \pm 7.3$ and it is 40 per cent.

The frequency of homogeneous groups surpasses the expected frequency by more than three times the cited standard deviation. Almost pure homogeneous groups of 11, 15 or 17 cells accentuate still further the tendency to cytologic homogeneity.

TABLE 5.—*Leukergy in Typhus Fever, Case 2, Eighth Day*

Counted number of scattered and agglomerated cells	Neutrophils	Lymphocytes	Monocytes	Total
	154 (72%)	46 (22%)	12 (6%)	212
In groups from 3 cells up	145 (94%)	20 (43%)	10 (83%)	175 (83%)
In groups from 5 cells up	145 (94%)	20 (43%)	7 (58%)	172 (81%)
Scattered cells	9 (6%)	26 (57%)	2 (17%)	37 (17%)

In table 5, which gives the counts in a case of typhus fever, one finds leukergy higher than in case 1 (83 against 57 per cent), with marked agglomeration of lymphocytes and monocytes (e.g., 43 against 14 per cent). Large groups from 5 cells up in the counted area are shown in table 6.

The lymphocytes formed homogeneous groups only: one of 7, and another of 13 cells, although only 11 per cent of the total number of cells clumped in groups of 5 or more were lymphocytes. This coincidence cannot be explained as purely fortuitous.

2. Dr. Steinhaus, professor of mathematics, University of Wroclaw, and Dr. Biernacki, professor of mathematics, University of Lubin, helped us in the statistical evaluation of our results.

In case 5 (table 9) a separate count of thirty major groups (from 5 cells upward) with 214 cells gave the picture shown in table 10.

TABLE 6.—*Composition of the Thirteen Larger Groups in Case 2*

	Neutrophils Only	Lymphocytes Only	Monocytes Only	Mixed Groups	Total Groups	Total Cells
Groups of 5 cells	2	..	1	..	3	15
Groups of 6 cells	..	..	..	1	1	6
Groups of 7 cells	2	1	..	..	3	21
Groups of 9 cells	..	..	..	1	1	9
Groups of 13 cells	..	1	..	..	1	13
Groups of 14 cells	1	..	..	..	1	14
Groups of 19 cells	1	..	..	..	1	19
Groups of 33 cells	1	..	..	..	1	33
Groups of 42 cells	1	..	..	..	1	42
Total	..	..	..	..	13	172

TABLE 7.—*Leukergy in a Rabbit After Injection of Killed Colon Bacilli, Case 3*

	Neutrophils	Lymphocytes	Monocytes	Total
Counted number of scattered and agglomerated cells	36 (19%)	141 (73%)	16 (8%)	193
In groups from 3 cells up	10 (29%)	41 (29%)	14 (87%)	65 (34%)
In groups from 5 cells up	6 (17%)	25 (17%)	12 (75%)	93 (22%)
Scattered cells	26 (72%)	100 (71%)	2 (13%)	128 (66%)

TABLE 8.—*Leukergy in Typhoid Fever, Third Week, Case 4*

	Neutrophils	Lymphocytes	Monocytes	Total
Counted number of scattered and agglomerated cells	114 (65%)	47 (27%)	14 (8%)	175
In groups from 3 cells up	103 (90%)	18 (38%)	10 (71%)	129 (74%)
In groups from 5 cells up	76 (66%)	13 (27%)	7 (50%)	94 (53%)
Scattered cells	11 (10%)	29 (62%)	4 (29%)	46 (26%)

TABLE 9.—*Leukergy in Fibrocaseous Pulmonary Tuberculosis, Case 5 (Temperature, 37.8 C. [100 F.]; Erythrocyte Sedimentation Rate, 96 mm. in First Hour and 120 mm. in Second Hour)*

	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Total
Counted number of scattered and agglomerated cells	168 (71%)	58 (25%)	5 (2%)	4 (2%)	235
In groups from 3 cells up	109 (65%)	21 (36%)	2 (40%)	3 (75%)	135 (57%)
Scattered cells	59 (35%)	37 (64%)	3 (60%)	1 (25%)	100 (43%)

TABLE 10.—*Composition of Thirty Major Groups in Case 5*

	Neutrophils Only	Lymphocytes Only	Monocytes Only	Mixed Groups	Total Groups	Total Cells
Groups of 5 cells	5	2	..	1	8	40
Groups of 6 cells	2	..	..	5	7	42
Groups of 7 cells	1	2	2	3	8	56
Groups of 8 cells	..	..	..	4	4	32
Groups of 10 cells	..	..	..	1	1	10
Groups of 12 cells	1	..	..	..	1	12
Groups of 22 cells	..	..	..	1	1	22
Total	..	..	..	..	30	214

Those groups contain 117 (54 per cent) neutrophils, 64 (29 per cent) lymphocytes, 30 (14 per cent) monocytes and 4 eosinophils. Nine groups are pure neutrophil groups of 5 or more cells, which makes 30 per cent, and the expected number would be (according to Dr. Biernacki):

$$f(p) = \frac{1}{30}(8p^5 + 7p^6 + 8p^7 + 4p^8 + p^{10} + p^{12} + p^{32})$$

where

$p = \frac{54}{100}$, which is 2.3 per cent.

The standard deviation is determined as follows:

$$\text{Standard deviation} = f^1(p)\sqrt{\frac{pq}{n}} < 1 \text{ per cent}$$

where

$f^1(p)$ is the derivative of $f(p)$, $p = \frac{54}{100}$, $q = \frac{46}{100}$ and $n = 214$

The result is that the theoretic frequency of pure neutrophil groups is 2.3 per cent $\pm$ 1, whereas the observed frequency is 30 per cent. A similar count shows that the theoretic frequency of pure lymphocytic groups is 0.09 per cent $\pm$ 0.07, and the observed frequency is 13 per cent. The tendency toward homogeneity of the groups is clear.

The clinical value of leukergy counts (percentage of agglomerated cells of each kind) has to be proved. Observations by clinicians interested in testing this new method of blood examination might be of importance.

The results of our preliminary examinations[3] may be summarized as follows:

Blood of normal persons and animals tested by the described method shows no agglomeration of leukocytes.

Different inflammatory stimuli and febrile diseases incite leukergy. It also was found in infants several weeks old.

Leukergy is not always accompanied by leukocytosis; it may be seen also in leukopenia.

It is not necessarily accompanied by fever; we see it in convalescents with normal temperature.

In some diseases with high erythrocyte sedimentation rates leukergy was only slight (chronic nephritis, neoplastic cachexia, panmyelophthisis). In others with marked leukergy the erythrocyte sedimentation rates were normal (convalescence after infectious diseases, febrile allergic states).

3. Fleck, L., and Borecka, D.: Ann. Univ. M. Curie Sklodowska **1**:335, 1946.

Irradiation with high doses of roentgen rays failed to incite leukergy in rabbits. The same observation was made after ultraviolet irradiation.

LEUKERGY AND PHAGOCYTOSIS

We assumed that leukergy may perhaps have an influence on phagocytosis. Experiments in rabbits failed to substantiate this view.

Immediately after the injection of bacteria which induce leukergy, the phagocytosis of injected bacteria diminishes. This negative phase is followed by a rise after a few days and the phagocytic index reaches a level higher than before the injection. The course of leukergy is

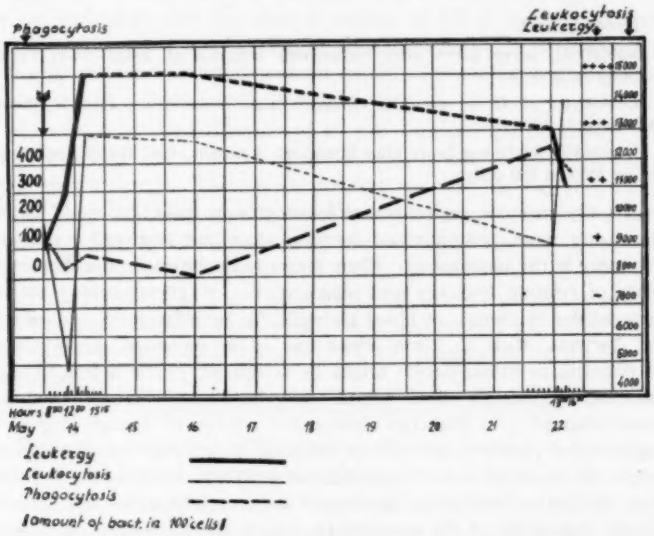


Fig. 2.—Leukocytosis, leukergy and phagocytosis (represented respectively by a thin black line, a heavy black line and a broken line) in a rabbit after intravenous injection of killed colon bacilli.

different: It rises several hours after the injection of bacteria and drops in a few days. Figure 2 illustrates one such experiment.

The negative phase of phagocytosis is probably due to the binding of opsonins by the injected antigen, according to the recent results of Boivin, Delaunay and Pages.[4] This follows from the observation that leukocytes studied during the negative phase when mixed with serum taken before the injection give a phagocytic index as high as that before

4. Boivin, A.; Delaunay, A., and Pages, J.: Bull. Acad. de méd., Paris **128**: 305, 1944.

the injection. The rise in phagocytic activity several days after injection is caused by the increase in the titer of opsonins. The following experiments may serve to illustrate these relations:

EXPERIMENT 1.

Rabbit 35, seven days after intravenous injection of killed colon bacilli: leukergy ++++.

Rabbit 37, normal: leukergy ±.

Leukocytes of 35 + serum of 35: 314 bacteria in 100 cells.

Leukocytes of 35 + serum of 37: 178 bacteria in 100 cells.

Leukocytes of 37 + serum of 37: 310 bacteria in 100 cells.

Leukocytes of 37 + serum of 35: 840 bacteria in 100 cells.

EXPERIMENT 2.

Rabbit 42, seven hours after intravenous injection of killed colon bacilli: leukergy + + + +.

Leukocytes of 42 + serum of 42 (both taken seven hours after injection): 74 bacteria in 100 cells.

Leukocytes 42 (seven hours after injection) + serum of 42 (before injection): 163 bacteria in 100 cells.

In any case the agglomerated leukocytes in leukergic blood do not show any more phagocytosed bacteria than the scattered leukocytes occurring in the same smear. There seems to be, however, another possibility of relating leukergy and phagocytosis: A great number of bacteria adhere to clumps of blood platelets, for in inflammation almost all the bacteria stick to these elements. The increased agglutination of platelets in inflammation might be compared to the action of antibacterial agglutinins which appear only later; bacteria are fixed and thus localized. As leukergic leukocytes adhere to a high degree to agglutinated platelets, an indirect influence of leukergy on phagocytosis might be assumed, i.e., if agglutinated platelets acted in the inflamed area as a factor facilitating the contact between leukocytes and bacteria. Under conditions of the experiment, where the number of bacteria is sufficiently high to provide easy contact, this effect cannot be seen.

It must be stressed, however, that the process of leukergy is independent of the presence of platelets. The following experiment may prove it:

A rabbit was given an injection of an antiplatelet serum obtained by immunizing a guinea pig with rabbit blood platelets. Ten minutes after the intravenous injection of 2 cc. of the antiplatelet serum smears of the rabbit's blood showed practically no platelets. The number before the injection was 30 per thousand red blood cells. Simultaneously the rabbit received intravenously a killed suspension of B. proteus X_{19} and the next day showed a high degree of leukergy. Microscopic examination revealed large groups of leukocytes free of platelets. At the time the number of platelets (these appeared gigantic in size in most cases) was 8 per thousand red blood cells. This slight rise was probably due to the inflammatory stimulus given by the injection of bacteria.

SOME FACTORS PROVOKING EXPERIMENTAL LEUKERGY

Leukergy may be easily induced in rabbits by injection (particularly intravenous injections) of gram-negative bacteria (B. coli, S. typhi, B. proteus X»). Approximately 150 millions of killed bacteria are sufficient. Gram-positive cocci (a strain of Staphylococcus albus, a strain of Staphylococcus aureus from a human abscess and a strain of Streptococcus hemolyticus) have been much less efficient. Attempts to produce leukergy by injections of killed Staph. aureus (up to 2 billion micro-organisms) failed. A suspension of live Staph. albus, however, injected into the knee joint was followed by purulent arthritis with leukergy. Injections of killed streptococci up to 500 millions had no effect on the leukergy. Not less than 2 billions of killed streptococci were needed to provoke leukergy. Killed diphtheria bacilli in amounts up to 500 millions were injected without effect. Two billions gave only a moderate and a short lasting leukergic effect.

Injections of the endotoxin of B. coli (antigen glycolipoidal) prepared in accordance with the technic of Boivin with trichloroacetic acid gave high leukergy. The hapten obtained by splitting the endotoxin [5] after boiling with acetic acid gave either no leukergy or at most a weak reaction, even though the material was injected in amounts twice or three times the original amount of endotoxin employed.[6]

Horse serum (1 to 2 cc.) gave in rabbits either no leukergy or only slight degrees, though in previously sensitized animals the reaction was sometimes strong, simultaneously with the appearance of the Arthus phenomenon.

Continued experiments can be summarized as follows:

Following perenteral administration of milk to men or animals, a transient leukergy of several hours' duration has been observed.

Heparin causes clumping of white blood cells in vivo and in vitro. The clumps show a tendency to form cytologically homogeneous groups, their homogeneity being much like that observed in leukergic blood. The phenomenon is now under investigation.

Furthermore, leukergy has been observed in the last five months of uncomplicated human pregnancy, persisting for some time after normal

5. The endotoxin and the hapten were controlled by precipitation with a specific serum. Rabbits given injections of the endotoxin show a rise in their respective agglutinating titers; rabbits given injections of the hapten failed to show a rise.

6. In comparison with the B. coli endotoxin the substance obtained from a strain of Staph. aureus by the method of Boivin with trichloroacetic acid was wholly ineffective.

delivery. Observations have been made on human pregnancy by Dr. Kwiatkowski and confirmed by us on pregnant rabbits.

Other kinds of bacteria or bacterial products will be investigated.

COMMENT

The most striking feature of leukergy is the cytologic selectivity of the process of cell clumping. The agglomeration itself can be seen at first sight in microscopic preparations, but the selectivity must be statistically confirmed to avoid any misleading formations of accidental groups. Statistical counts quoted show that the frequency of homogeneous groups surpasses by far the frequency of random agglomerations. This holds true as well for the agglomeration of neutrophil leukocytes, which is most common, as for eosinophils, lymphocytes or monocytes. It leads to the assumption that there are special mechanisms aggregating similar cells.

The study of different leukergy counts gives the impression that white blood cells of the kind which is just increasing in number are most susceptible to agglomeration, i.e., neutrophil leukocytes in increasing neutrophil leukocytosis, and lymphocytes in increasing lymphocytosis. Leukergy lasts approximately four days after a single stimulus (e.g., injection of killed bacteria).

The forces causing the selective agglomeration may be physicochemical in nature, or they may represent the special kind of physicochemical factors which are termed serologic forces. In the latter case leukergy would be a case of autoagglutination. The assumption of a serologic mechanism in leukergy is based on the fact that the homogeneous clumps consist of cells possessing the same specific, serologically distinguishable antigens, as stressed in a previous report. At present we may add the observation of a case of acute myeloid leukemia, in which myeloblasts formed separate groups, whereas more mature granulocytes, beginning with myelocytes, gave mixed clumps. A specific antigen of myeloblasts was found by Fleck and Lille[7] in 1940 and was confirmed in later studies by Steinberg and Martin.[8]

It must be emphasized, however, that other, nonserologic mechanisms cannot be excluded, since the formation of cytologically homogeneous agglomerations of white blood cells may be compared to the grouping of other blood cells—e.g., rouleaux formation of erythrocytes or clumping of thrombocytes, probably phenomena of a nonserologic type.

Our attempts to find differentiating features between leukergy and isoagglutination or heteroagglutination of red blood cells by means of different salt concentrations failed: a 2 per cent sodium chloride solution represses a high degree of leukergy and checks completely a slight one.

7. Fleck, L., and Lille, F.: Am. Rev. Soviet Med. **3**:174, 1945.
8. Steinberg B., and Martin, R. A.: J. Immunol. **52**:71, 1946.

A 4 per cent solution checks even a high leukergic effect. In general, the same holds true for isoagglutination of human red blood cells, and for the agglutination of rabbit white and red blood cells by either normal or immune antirabbit serum of rats and dogs.

Not all pyrogenic procedures have the same effect in producing leukergy; i.e., the injection of milk or foreign serum has either no effect or only a weak one. Foreign serum seems to act only on sensitized animals and even then not regularly. Injection of killed streptococci, staphylococci or diphtheria bacilli gives only slight leukergic reactions, in contrast to gram-negative bacteria, such as B. coli, S. typhi or B. proteus, which produce as a rule high degrees of leukergy. The antigen, glycolipoidal in nature, of colon bacilli prepared after Boivin's method gives the same positive result, but the hapten obtained by boiling this antigen with acetic acid does not produce marked leukergy. At present the question cannot be answered whether leukergy appearing after intrapleural injection of turpentine is due to the direct action of turpentine or to bacterial contamination of inflammatory foci in the lungs, which always have been found at autopsy.

The high occurrence of leukergy in infectious diseases and the regular appearance of leukergy in the experiment seems to allow us to look on it as on a phenomenon with a distinct role in pathogenesis. It has no direct relation to phagocytosis; the possibility of an indirect influence on phagocytosis through contact with thrombocytes was mentioned in an earlier paragraph. The influence on the migration of leukocytes, a problem of actual importance in the light of recent investigations of Boivin and Delaunay, will be studied.

SUMMARY

Leukergy, the phenomenon consisting in clumping of leukocytes in cytologically homogeneous groups, shows two kinds of cytologic selectivity:

1. The degree of agglomeration of various kinds of white blood cells is different; i.e., the percentage of clumped leukocytes, lymphocytes or monocytes varies in different cases. The clumping of neutrophils is most frequent. A differential method of evaluating the degree of leukergy is described.
2. The groups of cells show a remarkable cytologic homogeneity. This can be statistically proved.

Leukergy has no direct influence on phagocytosis. An indirect influence through the close contact of leukergic leukocytes with clumps of platelets, including bacteria, is discussed.

Leukergy can be positively induced by injection of gram-negative bacteria or their endotoxins. The effect of gram-positive bacteria is much weaker and less definite.

Increased salt concentration depresses the agglomeration of leukocytes, this action being similar to that on isoagglutination and heteroagglutination of blood cells.

CHANGES IN THE CAPSULE OF THE LYMPH NODE IN EXPERIMENTAL HYPERPLASIA

WILLIAM J. FURUTA, Ph.D.
CHICAGO

THE QUESTION as to how the capsule of the lymph node accommodates the enlargement of the organ during hyperplasia has not been answered. The solution may lie in one or more of the following possibilities: that the capsule, by means of proliferative changes, keeps pace with internal enlargement, that it is stretched passively as though it were an elastic sac, or that it disintegrates partially or totally and is later restored with new tissue which delimits the periphery of the enlarged organ.

The numerous publications concerning experimental and clinical observations of lymph node hyperplasia are concerned with the structural changes of the lymphatic tissues exclusive of the capsule. In morphogenetic studies the capsular changes have been mentioned by a few investigators, but as incidental findings. Gulland,[1] after making comparative studies of mammalian nodes, stated that the unequal peripheral growth of the cortical lymphatic tissue formed nodules protruding into the capsule, so that the segment of the latter between two points of evagination became a trabecula. Similar observations were reported by Kling[2] with regard to the developing nodes of human beings. Sabin,[3] in studying the development of lymph nodes in pig fetuses, concluded that the capsule impeded peripheral enlargement of the organ. She observed growth only at the margins where the capsule was still deficient. These points of view seem to indicate that the capsule, once formed, does not undergo any significant reconstruction throughout the growth of the organ.

The capsule, according to current views, is a fibrous structure which completely invests the node and through which the afferent lymphatic vessels enter the subcapsular sinus. It is said to be thickest at the hilus, where it ensheathes nodal blood vessels, nerves and efferent lymphatic vessels. From the inner surface of the capsule, including that of the hilus, strands of connective tissue pass into the organ as the trabecular framework. Although the thickness of the capsule varies with age and species, its structural components consist predominantly

From the Department of Anatomy, University of Illinois College of Medicine.

1. Gulland, G. L.: J. Path. & Bact. **2**:447, 1893.
2. Kling, C. A.: Arch. f. mikr. Anat. **63**:575, 1904.
3. Sabin, F.: Am. J. Anat. **4**:355, 1905.

of collagenous connective tissue fibers, with small amounts of reticular and elastic fibers. In certain animals (e.g., the cow), the capsules of the lymph nodes are especially thick and contain, in addition to the connective tissue component, an abundance of smooth muscle fibers.

In this paper, attention is focused entirely on the changes in the capsule of hyperplastic lymph nodes. Among the several laboratory animals convenient for experimentation the hamster was chosen first because its small nodes could be sectioned serially with economy of time and material. Later the rat was used because its nodes, although slightly larger, possess more definite and thicker capsules. Finally, normal and hyperplastic lymph nodes of calves were included in the study because the capsules of the nodes of these animals are the thickest and the most complex.

MATERIALS AND METHODS

Fifteen adult hamsters were given injections of Eberthella typhosus vaccine (0.1 cc. injected subcutaneously into each of the hindpaws). Four animals were killed after twenty-four hours; 4 were killed after seven days; in 7 animals the same dosage was repeated every three days, and the animals were killed ten days after the third injection. Four normal adult hamsters served as controls. The right and left popliteal and inguinal lymph nodes of both the vaccine-treated and the control animals were excised immediately after the killing.

Seventeen adult rats received 0.2 cc. of E. typhosus vaccine into each of their paws. Three were killed after nine hours, 4 after twenty-four hours, 2 after forty-eight hours and 8 after seventy-two hours. Seven normal adult rats served as controls. The right and left popliteal, inguinal and axillary lymph nodes of vaccine-treated and control animals were removed immediately after the killing.

Mediastinal and bronchial lymph nodes of 6 calves with acute pulmonary infections (bronchopneumonia) were obtained from the slaughterhouses through the aid of Dr. L. J. Cook, Meat Inspection Division, United States Department of Agriculture, Chicago. Mediastinal, bronchial and mesenteric nodes from healthy calves served as controls.

The specimens from hamsters and rats were fixed in 4 per cent formaldehyde solution, embedded in paraffin, sectioned serially at approximately 10 microns and stained with either hematoxylin-eosin or hematoxylin-picrofuchsin. The calf nodes, already fixed in formaldehyde solution when received, were prepared for histologic study in the same manner.

OBSERVATIONS

In general, the unfixed extirpated nodes of the vaccine-treated hamsters and rats appeared large and hyperemic. Since, however, nodes which appeared unusually large were occasionally found among the control specimens, a search for the possible significance of dimensional differences was not attempted. Furthermore, preliminary experience had revealed that microscopic differentiation was the most reliable, if not the only, means of determining whether or not hyperplasia was present.

Hamsters.—In the popliteal and inguinal nodes of normal hamsters several layers of fibroblasts dispersed between collagenous fibers formed a thin but intact capsule over the entire periphery of the organ. At the hilus, however, this

capsule became areolar and merged imperceptibly with the loose perinodal tissue. As a result, the boundary of the parenchyma at the hilus was not sharp, for here the perinodal tissue contained free lymphocytes that had migrated from the medulla. Sections stained with hematoxylin-picrofuchsin failed to show any muscular tissue in the capsule and the trabeculae.

The lymph nodes of vaccine-treated hamsters all exhibited hyperplasia of varying intensities. Even twenty-four hours after injection of the vaccine, large numbers of lymphocytes filled the sinuses, and the contrast between the cortex and the medulla so characteristic of the normal organ was effaced. Secondary nodules were not as yet present, and under low magnification the entire section of the node appeared as a dense, homogeneous mass which was markedly basophilic. The striking feature which typified these specimens was that the capsule not only was thin, being only the thickness of a single cell in places, but showed areas of disintegration. Figure 1 portrays a section of an inguinal node, in which the capsule overlying the cortex is distinct up to point *x*, but between this point and the hilus (*h*), the parenchyma merged directly with the perinodal adipose tissue (*f*). Free lymphocytes migrating peripherally into the latter produced a ragged cortical margin. In the hilar region the medulla sent out numerous small tongues of lymphocytes and reticular cells into the areolar tissue.

The hyperplastic changes just described were more pronounced in the nodes of hamsters killed seven days after the injection of vaccine. The areas in which cortical lymphocytic tissue protruded through open gaps in the capsule were more extensive. Both the extracapsular protrusions and the cortex proper had numerous pale-staining secondary centers in the lymphatic nodules. More marked, too, were the massive amounts of the medulla that projected into the areolar tissue of the hilus. In the perinodal adipose tissue just peripheral to the intact portions of the capsule there were occasional microscopic islands of lymphocytes, a feature which was not noticed in animals killed twenty-four hours after vaccination.

The nodes of hamsters which were given triple injections and killed ten days later showed essentially the same hyperplastic changes that were found in animals killed one week after a single dose.

Rats.—The normal somatic lymph nodes of the rat, except for their greater size, resembled those of the hamsters in their general histologic features. Although the capsule consisted of several tiers of fusiform fibroblasts as in the hamster, its thickness was about twice as great because of the increase of coarse collagenous fibers. Toward the hilus the compact capsule was again replaced by areolar tissue (fig. 2). This area was infiltrated by the medullary lymphatic tissue to a more pronounced extent than in the hamster, so that the determination of the external contour of the organ in the hilar region was difficult.

The nodes from the rats killed nine hours after injection of the vaccine appeared normal. In a few specimens the sinuses were filled with acidophilic coagulum which contained lymphocytes. The capsule was thin but intact, and the areolar tissue of the hilus contained small nests of reticular cells among the infiltrating lymphocytes.

With longer postinjection periods, the histologic changes simulated those which characterized the hyperplastic nodes of the hamsters. The extending of a portion of the cortex through a deficiency in the capsule was most prevalent in rats killed twenty-four hours after vaccination. Figure 3 shows a

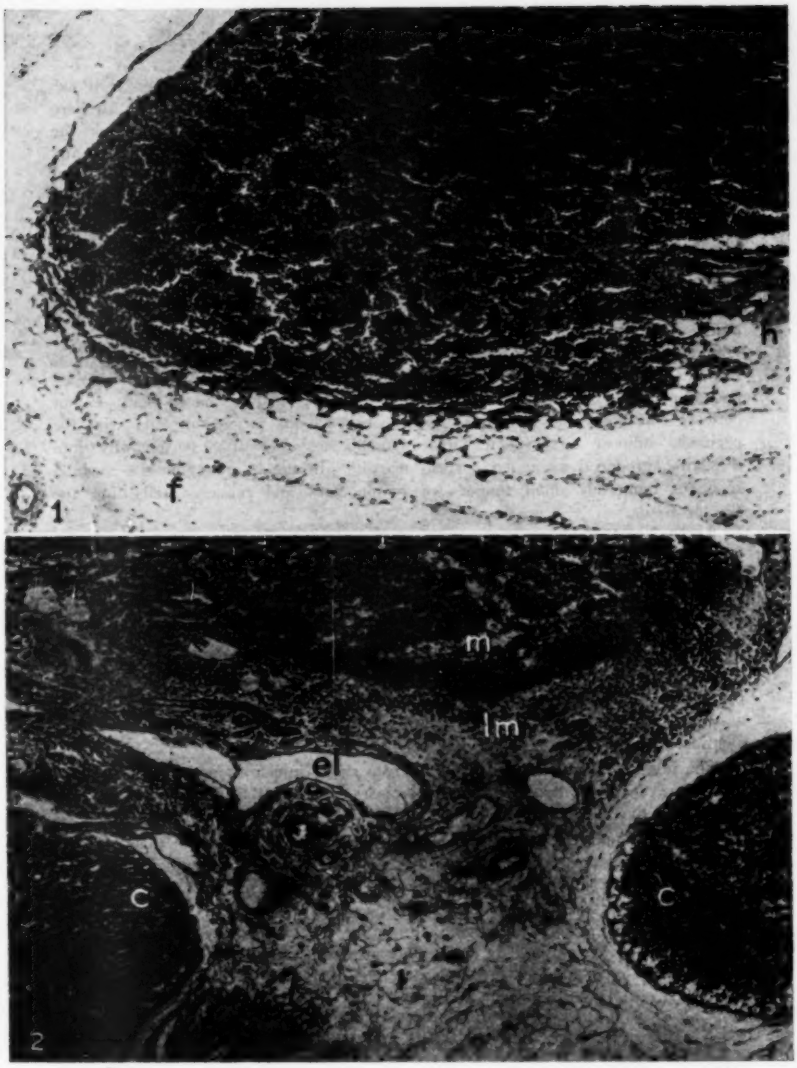


Fig. 1.—Section through a hyperplastic inguinal lymph node (adult hamster killed twenty-four hours after injection of typhoid vaccine); × 97. The capsule, *k*, which covers the pole of the node at the left ends at *x*. Note that between *x* and the hilus, *h*, the capsule is absent and the periphery of the cortex is in direct contact with perinodal areolar tissue, *f*.

Fig. 2.—Section through the hilar region of a normal popliteal lymph node (adult rat, control); × 95. *c*, indicates cortex; *h*, hilus; *el*, efferent lymphatic vessel; *m*, medulla; *lm*, lymphocytes infiltrating the hilus from the medulla. Note the contrast in sharpness between the peripheral margin of the cortex and that of the medulla.

Figures 1 to 6, inclusive, are unretouched photomicrographs.

section of a relatively small popliteal node in which approximately one quarter of the surface was devoid of capsule. The most peripheral extensions of the cortex consisted of lymphocytes (*l*) scattered diffusely in the perinodal fat. Centrally, however, there was a dense zone of reticular cells with abundant cytoplasm which resembled the primitive mesenchymal cells of a developing lymph node. Few lymphocytes were dispersed among them. The contrast between the eosinophilic reticular layer (*r*) and the basophilic cortex proper (*c*) was sharp, with a distinct boundary between the two (broken line in figure). The massive peripheral protruding of the medulla into the areolar tissue of the hilus was a common occurrence in these specimens.

In the hyperplastic nodes of rats killed forty-eight to seventy-two hours after vaccination, the morphologic contrast between the original cortex and the newly proliferated, extracapsular lymphatic tissue (indicated by arrows in fig. 4) was less, for the latter now contained an abundance of lymphocytes. The proliferated tongue possessed a sharp peripheral boundary, which was demarcated from the perinodal adipose tissue by a thin cap of fibroblasts lying parallel to the surface (*kl*). It is interesting to note that this newly formed covering was continuous above with the remnant of the original capsule (*k*) while below it joined the hilar areolar tissue. The hilus, however, still showed a diffuse scattering of medullary tissue.

Calves.—The normal visceral lymph nodes of calves are large oblong or bean-shaped bodies, which on the average measured about 2.5 by 1 cm. In overall histologic appearance they resembled the nodes of the hamster and the rat. The capsule, however, was thick and consisted chiefly of compact alternating strata of collagenous connective tissue and smooth muscle fibers. The latter were abundant also in both the coarse and the fine trabeculae of the cortex and the medulla. At the hilus the capsule broke up into an areolar structure in which connective tissue elements, smooth muscle and adipose tissue lay without definite arrangement. The outer margin of the medulla was again indistinct because the lymphocytes diffusely infiltrated the hilus.

Most of the bronchial and mediastinal lymph nodes of animals condemned at the abattoir because of bronchopneumonia displayed hyperplastic changes, while a few, despite their large size, appeared normal when examined microscopically. In the hyperplastic ones the outstanding manifestation was the presence of small areas of partial destruction of the capsule overlying the cortex. Yet unlike the hyperplastic nodes of hamsters and rats, these did not have cortical tissue protruding through minute breaks in the capsule. The typical appearance of incomplete loss of the capsule is shown in figure 5. The inner capsular surface was rough and scalloped, owing to a row of discrete spaces (*s*) which were lined with a single layer of flattened cells. The spaces found at the right of the figure were empty except for small amounts of coagulum and cell debris, while those toward the left communicated with the underlying subcapsular sinus and were packed with cells. The latter were lymphocytes and free reticular cells which extended peripherally from the congested subcapsular sinus (*ss*). It should be emphasized that the spaces just described were totally independent of the intracapsular afferent lymphatic channels as proved when they were traced through serial sections. The capsule here, as a result of undermining by the bayou-like arrangement, was about one-half the average thickness and also showed structural differences. Lymphocytes and reticular cells infiltrated between the layers of fibroblasts and muscle fibers and formed small intracapsular islands (*i*). Although not shown in the figure, the perinodal adipose tissue at this site contained similar accumulations.

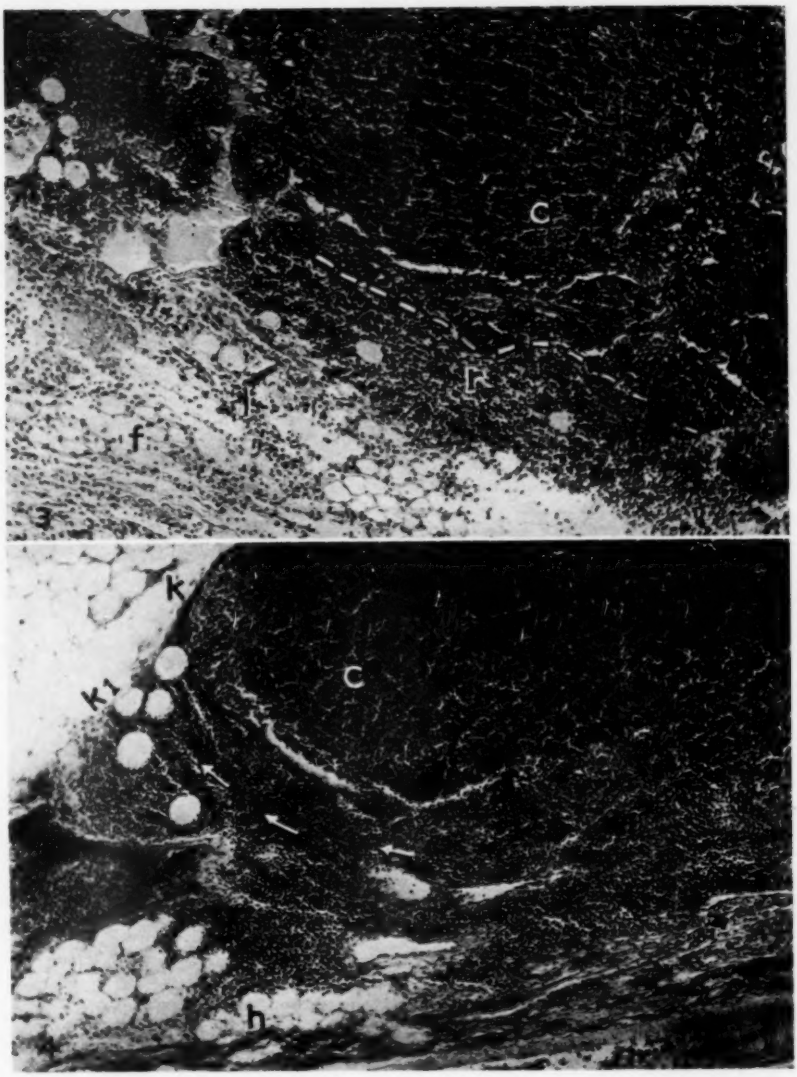


Fig. 3.—Section through an area of extracapsular protrusion of the cortex in a hyperplastic popliteal lymph node (adult rat killed twenty-four hours after injection of typhoid vaccine); × 95. *c*, indicates cortex; *f*, perinodal adipose tissue infiltrated by lymphocytes, *l*; *r*, solid mass of reticular cells and lymphocytes which is located just peripheral to a site of distintegration of the capsule. The broken line indicates the original boundary of the cortex.

Fig. 4.—Section through an area of extracapsular protrusion of the cortex in a hyperplastic axillary lymph node (adult rat killed seventy-two hours after injection of typhoid vaccine); × 95. *c*, indicates cortex; *k*, original capsule covering the surface of the cortex; *k1*, condensation of fibroblasts which forms a cap over the peripheral margin of newly proliferated lymphatic tissue; *h*, hilus of node diffusely infiltrated by lymphocytes. Arrows indicate the probable course of the extracapsular migration of the hyperplastic cortex.

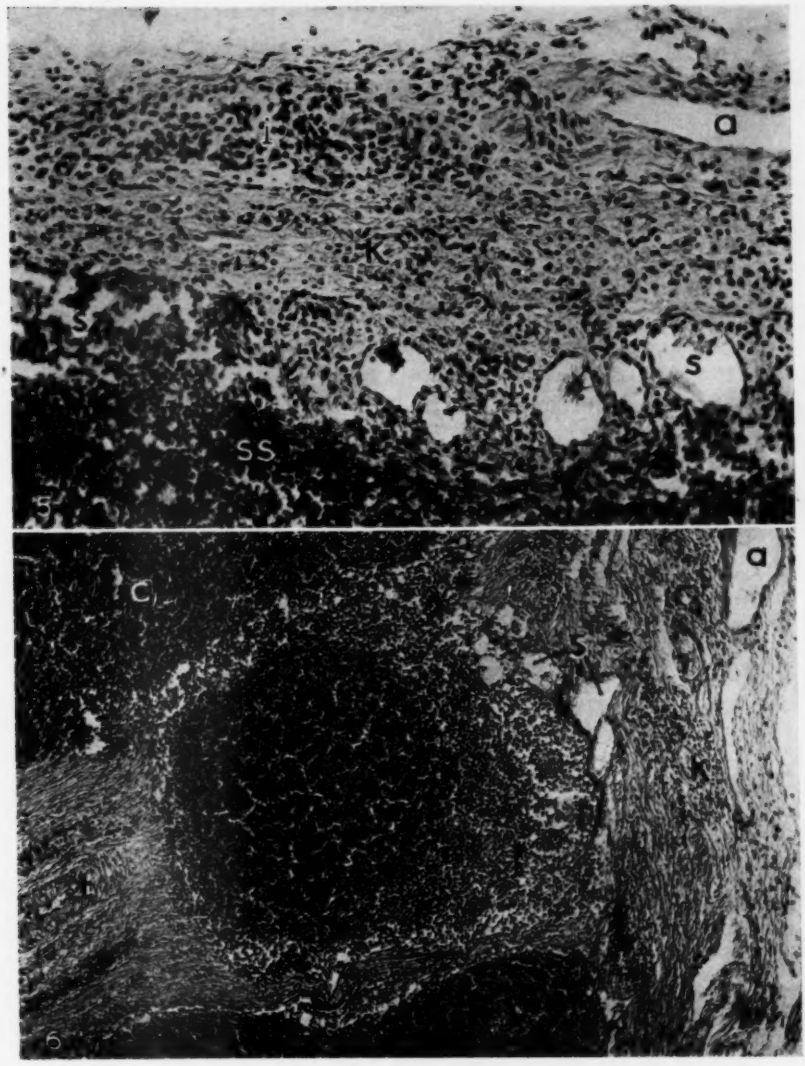


Fig. 5.—Section through an area of partial resorption of the capsule of a hyperplastic bronchial lymph node (calf condemned at abattoir for bronchopneumonia); × 200. *ss*, indicates a subcapsular sinus crowded with lymphocytes and free reticular cells; *s*, blind spaces lined with squamous epithelium; *k*, thinned capsule infiltrated by lymphocytes and reticular cells; *i*, intracapsular island of lymphocytes and reticular cells; *a*, afferent lymphatic channel.

Fig. 6.—Section through an area of trabecular and capsular resorption in a hyperplastic bronchial lymph node (calf condemned at abattoir for bronchopneumonia); × 90. *c*, indicates cortex; *k*, thinned capsule showing blind spaces, *s*, along its inner surface; *a*, afferent lymphatic channel. Note the solitary lymphatic nodule which occupies the site at which the capsule conneets with the trabecula, *t*.

The surfaces of the trabeculae situated adjacent to sites where the capsule was being eroded were also affected in a like manner (fig. 6). The section passes through the greatest diameter of a large solitary lymph nodule which had almost cut across the entire thickness of the trabecula. The thinned capsule, as well as the remaining body of the trabecula, was heavily infiltrated with lymphocytes and reticular cells.

Finally, the changes in the hilar region were almost identical with those observed in the hyperplastic nodes of hamsters and rats. The medulla extended into the hilus as dense cords and islands. Farther peripherally, the loose tissue filling the hilus contained diffusely scattered lymphocytes.

COMMENT

The possibility that the node enlarges in hyperplasia by breaking out peripherally through the capsule seems to be supported by the present experiments.

In the hyperplastic nodes of hamsters, the portions of the cortical lymphatic tissue protrude through the openings in the capsule and infiltrate the perinodal adipose tissue. Despite the range of postinjection periods (twenty-four hours to ten days) the results are fairly consistent, the slight difference being a quantitative one in which the degree of extracapsular migration of the lymphatic tissue increases with longer experimentation. There is no indication that a new capsule is formed around the protruded mass. At the hilus of the normal node of the hamster the capsule becomes loose and merges with the areolar tissue. Even in the normal node, lymphocytes of the medulla diffusely infiltrate the hilus to some degree. It is here that the maximum peripheral expansion of the node is noticed in hyperplasia, for the areolar tissue offers probably less resistance than the capsule over the cortical surface.

The findings are almost duplicated in the hyperplastic lymph nodes of rats, although the capsule of the normal organ of this animal is somewhat thicker than that in the hamster. Again the greatest amount of peripheral enlargement occurs in the areolar tissue of the hilar region. A feature of the specimens from the rats which was not encountered in the hamsters is the quality of the structural changes in the capsule over the cortex incident with the duration of the postinjection period. In nodes of animals killed twenty-four hours after vaccination the protruded lymphatic tissue lies in direct contact with the perinodal fat, but in the animals killed after forty-eight to seventy-two hours such areas show the beginning of what appears to be a new capsule which delimits the extent of peripheral hyperplasia. This probably signifies a cessation of further enlargement and a restoration or repair of the damaged part of the capsule. The fibroblasts of the new segment are derived from the indifferent cells of either the perinodal adipose tissue or the reticular tissue of the cortex.

Thus, in nodes of hamsters and rats, acute hyperplasia means that portions of the cortex have penetrated through the thin capsule and the medulla has spread peripherally into the hilus. Consequently, the enlarged node possesses a rough, "bumpy" external contour over the cortical surface, while the familiar indentation at the hilus is mostly obliterated.

In the calf's lymph node, the capsule of which is much thicker, capsular rupture apparently does not occur, at least in the hyperplastic material observed. Areas are found, however, in which the capsule is eroded along its inner surface with consequent thinning. Lymphocytes and free reticular cells emigrate from the subcapsular sinus beyond the area of erosion, as witnessed by numerous intracapsular and extracapsular islands of these cells. That the capsule and the trabeculae are labile and subject to modification under proper stimulus is shown by the severe trabecular resorptions. To explain the absence of extracapsular projections of the cortical lymphatic tissue of the hyperplastic nodes of calves tempts one to indulge in speculations. Has the node enlarged by commensurate interstitial proliferation of the capsule? Since the material obtained from the slaughterhouse gives no clue as to the time of initiation or the duration of the hyperplastic changes, the chance that these specimens exhibit terminal stages in which capsular damages have been repaired must be considered. Over the medulla at the hilus the enclosure is completed by a loose structure, and it is at this site that pronounced peripheral enlargement of lymphatic tissue takes place in hyperplasia.

That acute hyperplasia of the node involves passive stretching of its covering seems to be a minor consideration, especially when the structural features of the capsule are appreciated. It may be parenthetically stated that the general question as to how a saclike structure, whether it be a capsule of a viscus or the skin of the body surface, responds to fluctuations of the masses contained within has not received a satisfactory explanation.

SUMMARY

The normal somatic lymph nodes of hamsters and rats possess a capsule which is relatively thin, compact and devoid of smooth muscle. At the hilus the capsule is areolar and contains lymphocytes which have infiltrated it from the medulla of the node. In hyperplasia the capsule shows areas of disintegration through which portions of the cortex extend out into the perinodal adipose tissue. Simultaneously large amounts of the medulla migrate peripherally as cords and islands into the surrounding areolar tissue of the hilus.

The capsules of the normal visceral lymph nodes of calves are extremely thick and contain abundant smooth muscle. However, at the hilus the medulla is covered by a loose structure which resembles areolar tissue, except that it exhibits strands of scattered smooth muscle, and contains lymphocytes that have emigrated from the medulla. In the hyperplastic nodes the capsule of the cortex is resorbed in certain areas from its inner surface. The portion of the capsule just peripheral to the resorption is thin and contains small islands of lymphocytes and reticular cells. The destructive process also affects any trabecula located near the site of capsular erosion. Disintegration involving locally the entire thickness of the capsule has not been found. The hilar region shows a massive migration of medullary parenchyma which extends into the surrounding loose tissue.

DISTRIBUTION OF THE LYMPHATICS OF THE HUMAN KIDNEY AS SHOWN IN A CASE OF CARCINOMATOUS PERMEATION

ARNOLD J. RAWSON, M.D.
PHILADELPHIA

ALTHOUGH the lymphatic channels of the kidney have been studied by a number of investigators, and the distribution of the main channels is fairly well known, there is still no general agreement concerning the distribution of the smaller channels. This lack of agreement may be attributed to technical difficulties. Hitherto, with one exception, the distribution of the renal lymphatic channels has been traced by means of injections of pigment. As pigment introduced into the hilar trunks by retrograde injections is usually unable to pass the valves, the most successful studies have been those employing subcapsular injections, particularly in living animals. It is apparent, however, that any forceful injection of pigment will tend to produce artefacts, which may make morphologic interpretation difficult and uncertain. The one study which did not involve injection of pigment was that of Vogel,[1] who studied a kidney in which the lymphatic channels were distended with carcinoma cells. It cannot be denied, however, that artefacts may likewise occur in such preparations.

The principal points of disagreement are concerned with the relations existing between the lymphatic channels and the glomeruli, the medulla and the medullary rays. With regard to the glomeruli, some authors [2] have believed that lymphatic vessels accompany the afferent and efferent arterioles, while others [3] have denied this; some [4] have believed that lymphatic channels enter the glomerulus, but others [5] have

From the Laboratory of Pathology, School of Medicine, University of Pennsylvania.

1. Vogel, L.: Virchows Arch. f. path. Anat. **125**:495, 1891.

2. (*a*) Rindowski, T., cited by Pierce.[3a] (*b*) Kumita: Arch. f. Anat. u. Physiol., 1909, p. 99.

3. (*a*) Pierce, E. C., II.: Anat. Rec. **90**:315, 1944. (*b*) Vogel.[1]

4. (*a*) Maximow, A. A., and Bloom, W.: A Textbook of Histology, ed. 3, Philadelphia, W. B. Saunders Company, 1938. (*b*) Ssysganow, A. N.: Ztschr. f. d. ges. Anat. **91**:770, 1930. (*c*) Rindowski.[2a] (*d*) Kumita.[2b]

5. (*a*) Stahr, H.: Arch. f. Anat. u. Entwcklngsgesch., 1900, p. 41. (*b*) Kutsuna, M.; Kiyozumi, M., and Yamasita, S., cited by Pierce.[3a] (*c*) Drinker, C. K., and Yoffee, J. M.: Lymphatics, Lymph, and Lymphoid Tissue, Harvard University Monograph in Medicine and Public Health No. 2, Cambridge, Mass., Harvard University Press, 1941. (*d*) Pierce.[3a]

been in disagreement. Some [6] have demonstrated the presence of extensive lymphatic networks about Bowman's capsule, whereas others [7] have failed to find them. With regard to the medulla, some authors [8] have reported the presence of lymphatic networks, while others [7] have asserted that lymphatic channels do not occur in this location. The same question arises concerning the presence or the absence of lymphatic channels in the medullary rays. In view of this controversy it seems worth while to trace the distribution of lymphatic channels in a human kidney obtained at a postmortem examination in a case in which extensive carcinoma had prominently permeated the lymph channels.

METHODS

The kidney studied was from a patient at the Hospital of the University of Pennsylvania who was found to have a large ulcerated adenocarcinoma in the cardia of the stomach, on the lesser curvature just below the esophagogastric junction. There was extraordinarily widespread lymphatic permeation extending to the kidneys, lungs, pancreas, adrenal glands and retroperitoneal tissues; the liver was not involved. No tumor nodules had formed; at all sites the neoplastic cells were confined to the lymphatic vessels.

The kidneys were bisected in the usual manner. As there was no gross evidence of carcinomatous involvement, the capsules were stripped from them to allow examination of the surface; hence the capsular lymphatic channels could not be included in the present study. Microscopic examination of routine sections revealed that the lymphatic vessels were made clearly visible because they were distended by seemingly solid cords of tumor cells within their lumens. The walls of the lymphatic channels and their endothelial lining were clearly demonstrated.

It was recognized, of course, that channels might exist of such small caliber that carcinomatous permeation might not be permitted. This study, therefore, is of necessity limited to the tracing of those channels which were large enough to contain carcinoma cells. In order to trace the course of the lymphatic vessels in detail, two longitudinal and two sets of consecutive coronal wedge-shaped blocks were cut as shown in fig. 1 *A*. Several sections were prepared from each of the coronal blocks, and serial sections 5 microns in thickness were made from the longitudinal blocks. Fifty consecutive sections were cut from each of the latter, each section being examined. All tissues were stained with hematoxylin and eosin; the neoplastic cells were conspicuous because of their large size and basophilic affinity.

RESULTS

The distribution of the lymphatic channels of the human kidney examined is diagrammatically shown in figure 2. All the demonstrable lymphatic vessels are in close approximation to the arterial and venous channels, except the afferent and efferent arterioles and the glomeruli,

6. Vogel.[1] Rindowski.[2a] Kumita.[2b] Ssysganow.[4b]
7. Stahr.[5a] Kutsuna and others.[5b] Pierce.[3a]
8. Rindowski.[2a] Kumita.[2b] Ssysganow.[4b]

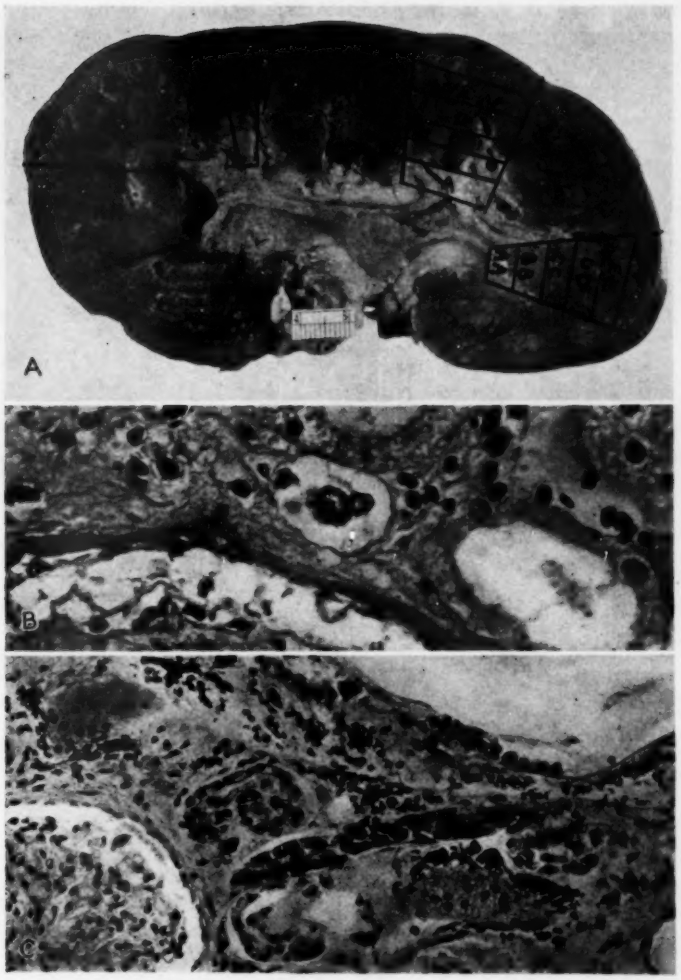


Fig. 1.—*A*, location of blocks of tissue studied. Sections *A* to *G* (inclusive) and *AA* to *FF* (inclusive) are coronal blocks. Sections *H* and *HH* are longitudinal blocks.

B, small lymphatic vessel lying just outside of Bowman's capsule. Serial sections show that the vessel begins here as a blind channel and continues as a short unbranched segment which communicates with the perivascular lymphatic networks (see fig. 3 *C*). Note the close, but chance, relation of the lymphatic vessel to a convoluted tubule. Hematoxylin and eosin; × 547.

C, lymphatic vessel appearing to enter the glomerulus at the lower left. Serial sections, however, show that it actually passes around a small arc of Bowman's capsule, and is part of an extensive network about the interlobular vein at the upper right. Hematoxylin and eosin; × 265.

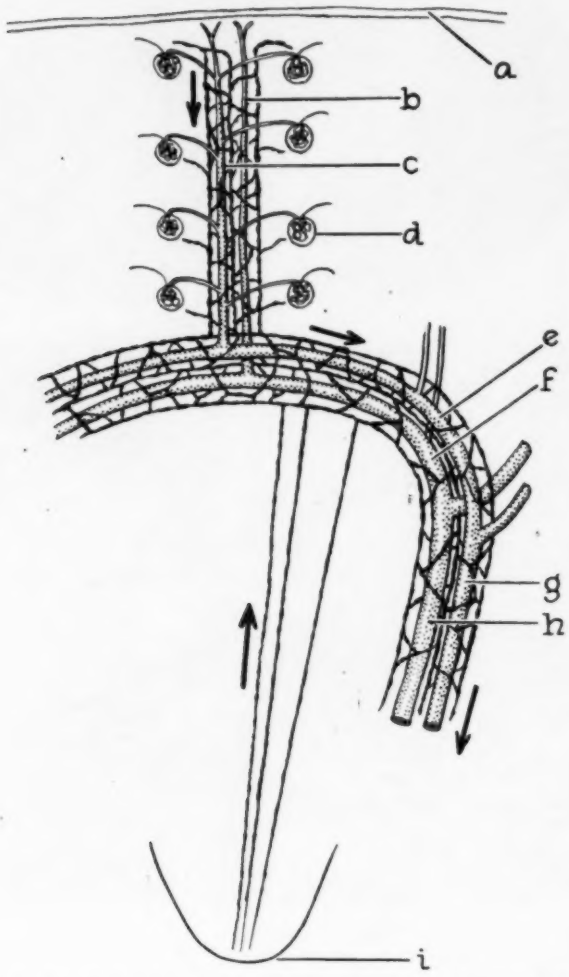


Fig. 2.—Lymphatic channels of the human kidney (diagrammatic). Two separate systems are demonstrable. One begins in the cortex and accompanies the interlobular vessels toward the corticomedullary junction; the other starts at the papilla and ascends to join the cortical system at the corticomedullary junction. From there large trunks follow the arcuate and interlobar vessels to leave the kidney at the hilus. Arrows show the probable direction of the lymph flow. The structures shown are: (*a*) tunica fibrosa, (*b*) interlobular vein, (*c*) interlobular artery, (*d*) glomerulus, (*e*) arcuate artery, (*f*) arcuate vein, (*g*) interlobar artery, (*h*) interlobar vein and (*i*) papilla.

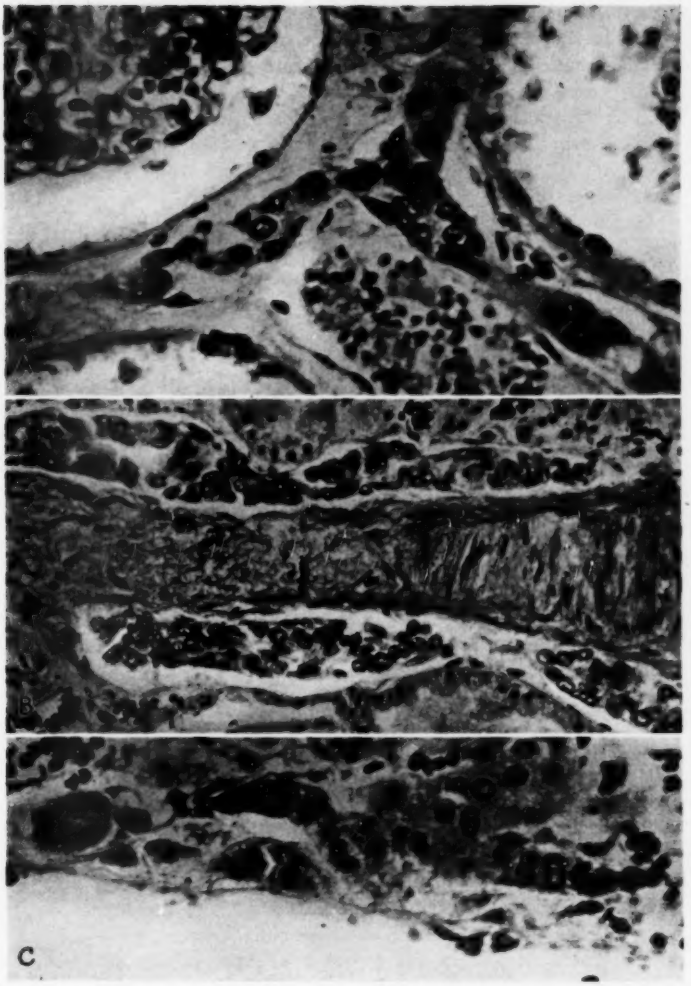


Fig. 3.—*A*, lymphatic vessel (cut longitudinally) in close contact with Bowman's capsule and forming a short arc about it. Serial sections show that this is actually a segment of a loose perivenous network accompanying an interlobular vein. Hematoxylin and eosin; × 547.

B, two prominent lymphatic vessels lying on either side of an interlobular artery. The vessels have been cut longitudinally. Note the absence of lymphatic channels around the intertubular capillaries. Hematoxylin and eosin; × 274.

C, small lymphatic channels forming a network about an interlobular vein in the outer half of the cortex. Hematoxylin and eosin; × 547.

which in the preparations are not accompanied by lymphatic vessels. The lymphatic channels are decidedly more plentiful in the cortex than in the medulla.

Two separate systems of channels are demonstrable, each of which can be shown to begin blindly by the abrupt appearance in serial sections of a lymphatic vessel which then can be traced as a single channel. Farther on such a channel anastomoses with others, which enlarge progressively. One system of lymphatic channels has its beginning as tiny blind-ending vessels which lie in close contact with Bowman's capsule (fig. 1 *B*). These channels gradually enlarge and form nets around both the arterial and the venous vessels of the cortex, beginning near the terminal branching of both arteries and veins (no lymphatic vessels are demonstrable about the stellate veins); however, no lymphatic network is demonstrable in these sections around the afferent or the efferent arterioles of the glomeruli, and none penetrate through Bowman's capsule (fig. 1 *C*). The latter may occasionally be partly surrounded by segments of nets which wind loosely about adjacent vessels (fig. 3 *A*); these lymphatic arcs are part of the perivascular network, however, and are only incidentally related to Bowman's capsule. The intertubular capillaries, likewise, have no demonstrable network of lymphatic channels; lymphatic channels winding loosely about the arteries and veins do, however, frequently come in chance contact with a convoluted tubule (fig. 1 *B*). The lymphatic nets about the cortical arteries and veins are prominent; those of the arteries are, on the average, somewhat larger and run a straighter course (fig. 3 *B*). Especially noteworthy are the lymphatic vessels which lie in close proximity to the large thin-walled venous channels that are prominent particularly in the outer half of the cortex (fig. 3 *C*). The anatomic connection of those venous "sinuses" is not clearly known; in the serial sections studied they appear to be parts of the interlobular veins, which in this region are exceptionally large and thin walled. Accompanying the interlobular vessels (fig. 4 *A*), the lymphatic channels progress toward the hilus, winding around the arcuate vessels (fig. 4*B*) and interlobar arteries and veins, and, finally, leaving the kidney at the hilus to terminate in the nodes on either side of the aorta.

Another system of lymphatic channels begins blindly as a network beneath the mucosa of the papilla (fig. 5 *A*); lymphatic channels from this region ascend in a more or less straight line, gradually increasing in size, running parallel to the small blood vessels of the medulla (fig. 5 *B*) and emptying into the larger lymphatic channels which surround the arcuate arteries and veins. Because of the opposing directions, with respect to increase in size of the lumen, which characterize the two groups of lymphatic vessels described, they are considered to be separate systems.

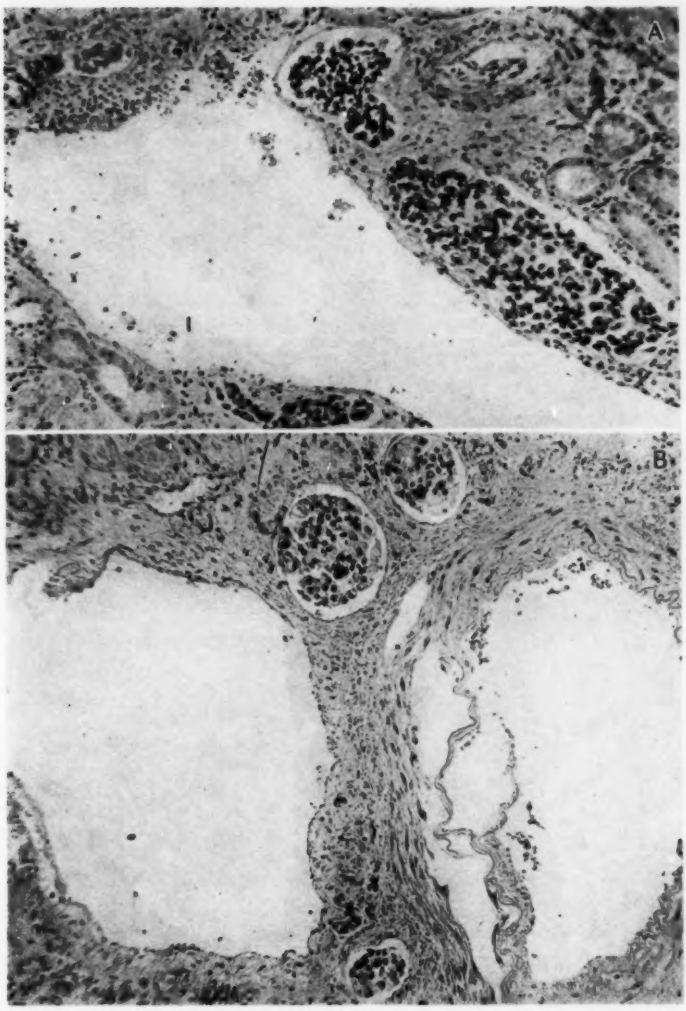


Fig. 4.—*A*, interlobular vein (near the corticomedullary junction) about which lymphatic channels are forming a rich network. Hematoxylin and eosin; × 91.
B, large lymphatic trunks accompanying arcuate vessels. Hematoxylin and eosin: × 108

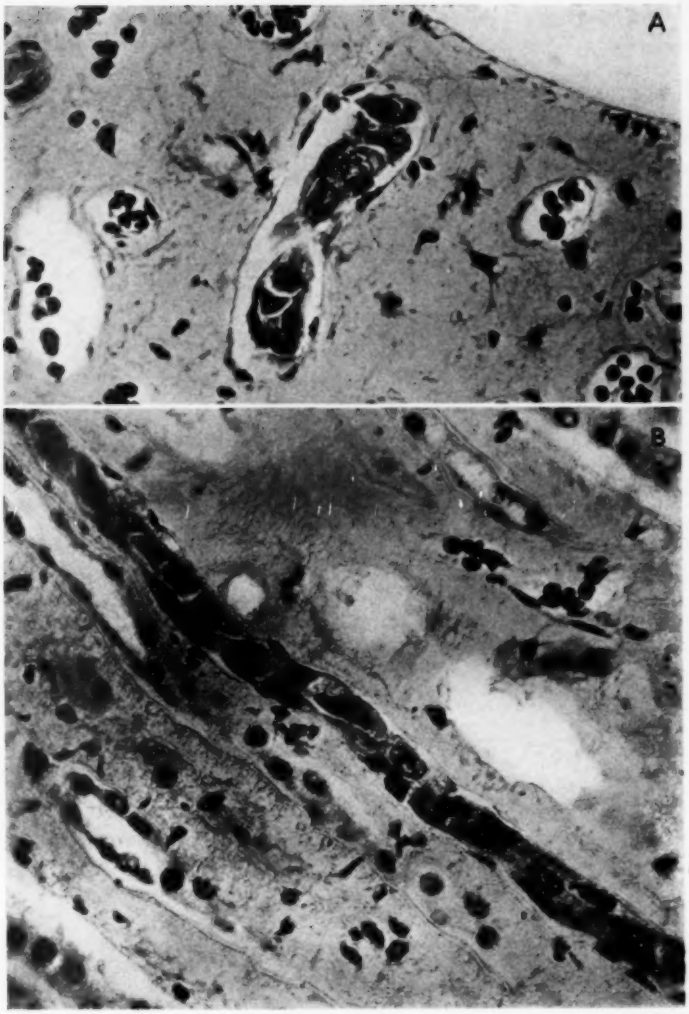


Fig. 5.—*A*, lymphatic vessel in a papilla. Serial sections show that this channel begins blindly beneath the mucosa; it passes through the medulla, running parallel to collecting tubules and blood vessels to terminate in the large lymphatic trunks about the arcuate vessels. Hematoxylin and eosin; × 522.5.

B, medullary lymphatic vessel running parallel to tubules and blood vessels. The vessel starts as a blind end at the papilla and empties into the lymphatic trunks about the arcuate vessels. Hematoxylin and eosin; × 497.5

COMMENT

Trueta and his co-workers [9] have recently offered convincing evidence for the existence of two hemic circulations, a greater and a lesser, in the kidney. Both take their origin from, or close to, the arcuate arteries. The greater circulatory pathway for blood consists of the interlobular arteries, afferent arterioles, glomerular capillaries, efferent arterioles, capillaries of the medullary rays, capillaries about the convoluted tubules, collecting veins and interlobular veins. The lesser circulatory pathway for blood consists of the afferent arterioles of the juxtamedullary glomeruli, these glomeruli themselves, their efferent arterioles and the vasa recta, arterial and venous, of the medulla. The studies here presented seem to indicate a double lymphatic system which parallels the venous component of this double hemic system. The greater lymphatic system, that of the cortex, accompanies the greater hemic system. The lesser lymphatic system, that of the medulla, appears to follow the course of the vasa recta of the lesser hemic system. The direction of flow in the lymphatic channels appears to be the same as that of the venous stream.

It has been shown by Schmidt and Hayman [10] that increased renal blood flow, in addition to augmenting urinary output, will increase the lymph flowing from the kidneys. It would thus seem that the renal lymphatic vessels may have an important regulatory function, preventing too much fluid from being excreted, and also preventing the accumulation of fluid in the kidney itself. The close relationship between the wide, thin-walled veins of the outer half of the cortex and the numerous fine lymphatic channels which form a network about them suggests the possibility that an exchange of fluid may take place in this region.

SUMMARY

The lymphatic channels of the human kidney have been traced in a case in which permeating carcinoma cells made these channels easily visible.

It was found that the lymphatic channels begin blindly in two locations, namely, closely adjacent to the capsule of Bowman, and beneath the mucosa of the papilla. From these origins two networks arise which accompany the arterial and the venous vessels of the kidney. The network arising in the medulla drains upward, toward the arcuate vessels, whereas that beginning near Bowman's capsule drains in the opposite direction. The two become confluent about the arcuate vessels.

9. Trueta, J.; Barclay, A. E.; Daniel, P. M.; Franklin, K. J., and Prichard, M. M. L.: Studies of the Renal Circulation, Springfield, Ill., Charles C Thomas, Publisher, 1947.

10. Schmidt, C. F., and Hayman, J. M., Jr.: Am. J. Physiol. **91**:157, 1929.

No lymphatic channels are demonstrable in the glomeruli, about the afferent or the efferent arterioles, or about the intertubular capillaries. If such networks exist, their caliber is too small to permit carcinoma cells to permeate them.

Especially noteworthy is the close association of the lymphatic channels and the large thin-walled veins of the outer half of the renal cortex. It is believed that these veins are expansions of the interlobular veins and their tributaries and that the closely approximated lymphatic and venous channels may form a regulatory system for fluid exchange.

Case Reports

MICROCYSTIC (THYROID-LIKE) KIDNEY

HAROLD W. VOTH, M.D.
WICHITA, KAN.

THYROID-LIKE dilatation of renal tubules is largely regarded as a result of pyelonephritis.[1] This opinion is so general that few original articles have been written on the subject.

A case is now reported, first, because no description of similar extensive dilatation of tubules could be found in the literature and, second, because this case raises serious doubt whether thyroid-like kidneys are always due to pyelonephritis.

REPORT OF A CASE

A 22 year old Negro woman was admitted to the hospital on Nov. 29, 1947. She complained of stiffness and soreness of all joints, a condition which had been present for the past year. The patient had been seen by a physician in Oklahoma during the course of her illness, but information relating to the diagnosis and the medication was not available. For the past three weeks the patient had observed that her arthritic symptoms were becoming more severe and that marked lassitude had developed.

Examination revealed an emaciated, drowsy Negro woman. There was fusiform swelling of the interphalangeal proximal joints with stiffness of elbows and knees. The pulse rate was 96; the temperature, 97 F.

A high white cell count suggested infection, and 50,000 units of penicillin was given every three hours. The patient was drowsy and semicomatose throughout her hospitalization. She underwent minor convulsions and died in apparent uremic coma two days after admission.

The Wassermann and Kline tests of the blood showed 4 plus reactions. The specific gravity of the urine was 1.010; the urine contained albumin (2 plus), occasional red blood cells and clumps of white blood cells. The hemoglobin was 6.1 Gm. per hundred cubic centimeters. The white cell count was 20,000, and the differential count showed segmented forms (67 per cent), band forms (22 per cent) and lymphocytes (10 per cent).

Postmortem inspection revealed a well developed but emaciated Negro woman. There was slight swelling of the proximal joints of the fingers. No edema, jaundice or lesions of the skin were visible. The pericardial sac contained 8 cc. of serofibrinous fluid. The right auricle and ventricle were flabby. There was hypertrophy of the left ventricle. Each kidney weighed 160 Gm. and measured 11.5 by 6 by 4 cm. The capsule stripped with ease. The surface was pale grayish brown and peppered with minute rounded opaque areas 1 mm. in diameter. No petechiae were visible. There was indistinct shallow lobulation with several shallow scars. Section revealed a pale grayish brown cortex with honeycombed structure. The orderly arrange-

From the Department of Pathology, St. Francis Hospital.

1. (*a*) Boyd, W.: Canad. M. A. J. **47**:128, 1942. (*b*) Weiss, S., and Parker, F., Jr.: Medicine **18**:221, 1939. (*c*) Mallory, G. K.; Crane, A. R., and Edwards, J. E.: Arch. Path. **30**:330, 1940.

ment of the renal pyramids with the apexes converging toward the renal sinus was slightly distorted, for in one area the renal columns of Bertini were prominent, overshadowing the adjoining pyramid, while in another pyramid the apex was directed into the calix laterally (fig. 1). Calices, pelves and ureters showed no dilatation or gross abnormalities.

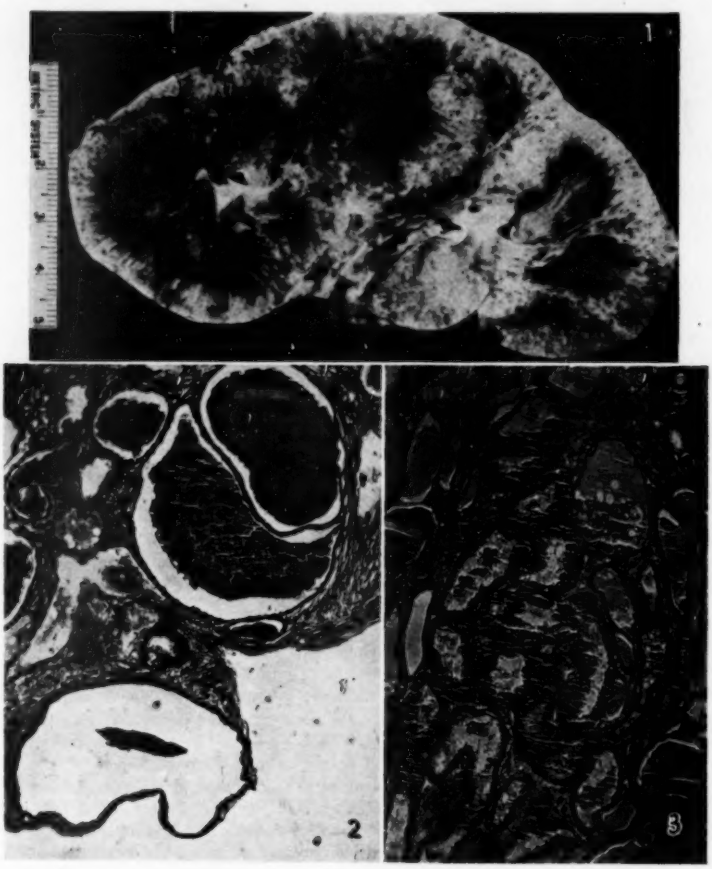


Fig. 1.—Longitudinal section of the microcystic kidney. Note macroscopic cysts and slight distortion of structure.

Fig. 2.—Dilated tubules protruding into the calix; × 100. There is little inflammatory reaction in the papillae.

Fig. 3.—Degenerative changes in tubules; × 100.

Microscopically, the majority of the glomeruli revealed a dilated capsule filled with a light pink homogeneous material. The glomeruli revealed varying changes. Many were set off by pericapsular fibrosis. The ones surrounded by dense fibrous

tissue were shrunken, and different stages of obliteration with early hyalinization were apparent. No epithelial crests were visible. Most of the glomeruli showed tufts that varied in size, some being large and cellular, others revealing only a small cellular mass at the periphery as though compressed by the surrounding fluid.

Nearly all the tubules appeared large and contained a pink-staining "colloid" of different densities. The majority of the tubules of both cortex and medulla showed enormous cystlike dilatations lined by a single layer of flattened epithelium. Many dilated tubules could be followed for some length without interruption. Others showed the wide cystlike spaces separated by thin septums or partial septums, the cysts lying adjacent to each other in a linear fashion and extending for great lengths in the cortex, medulla and papillae. Many cross sections through the cortex, medulla and papillae showed similar cystlike structures. Tubules that did not present the enormous cystlike dilatations were generally larger than normal and had a well defined epithelium; these also contained the "colloid," deep red–stained material. Small, apparently normal tubules were found in the sections. Occasional polymorphonuclear leukocytes were noted in the tubules. Cloudy swelling and varying stages of degeneration of the tubules, particularly the proximal convoluted ones, was noted.

The medulla revealed an increased amount of loose vascular stroma with scattered areas of round cell infiltration and a sparse scattering of leukocytes. The connective tissue and round cell infiltration of the cortex was more diffuse, dense and prominent. The epithelium of the calices and pelves was of the transitional type, with no thickening or metaplasia. The blood vessels showed no intimal or medial thickening.

COMMENT

Doubt as to whether inflammation was the cause of this unusual microcystic kidney arose from the observation that there was no chronic papillitis or fibrous barrier in the medulla. Staemmler and Dophiede,[2] in their description of chronic contracted pyelonephritic kidneys, stated that there is slowly progressive destruction of the cortex, terminating in a thyroid-like tissue, due largely to the productive medullary inflammation, which in turn causes fibrosis of the pyramids. This fibrosis forms a barrier near the corticomedullary junction and produces obstruction of the tubules with resultant thyroid-like cystic dilatation.

While in this case inflammation was present in both cortex and medulla, it apparently had not interfered with the continuity of the tubules and the pelvis. Figure 2 shows that small cysts may protrude into the pelvis with no fibrous tissue separating pelvic epithelium and cysts. These kidneys showed definite inflammatory and degenerative changes, but the changes were confined largely to the cortex. Tubular degeneration with swelling oxyphilic epithelial staining and occasional loss of nuclear stain are seen in figure 3. Round cell infiltration, increase of fibrous tissue and all stages of degeneration of glomeruli appeared. Figure 4 reveals one of the characteristic glomeruli with surrounding inflammatory changes.

Polymorphonuclear leukocytes in small numbers were present in a few of the collecting tubules. However, since most of the tubules

2. Staemmler, M., and Dophiede, W.: Virchows Arch. f. path. Anat. **277**:713, 1930.

contained colloid-like material without leukocytes, Mallory's [1c] view that the material is derived from imprisoned disintegrating leukocytes is questioned.

Varying densities of the albuminous material were observed in nearly all the tubules and most of the glomeruli. Thus, the theory of Staemmler and Dophiede [2] that the material is a product of atrophic tubular epithelium does not explain the findings in this case.

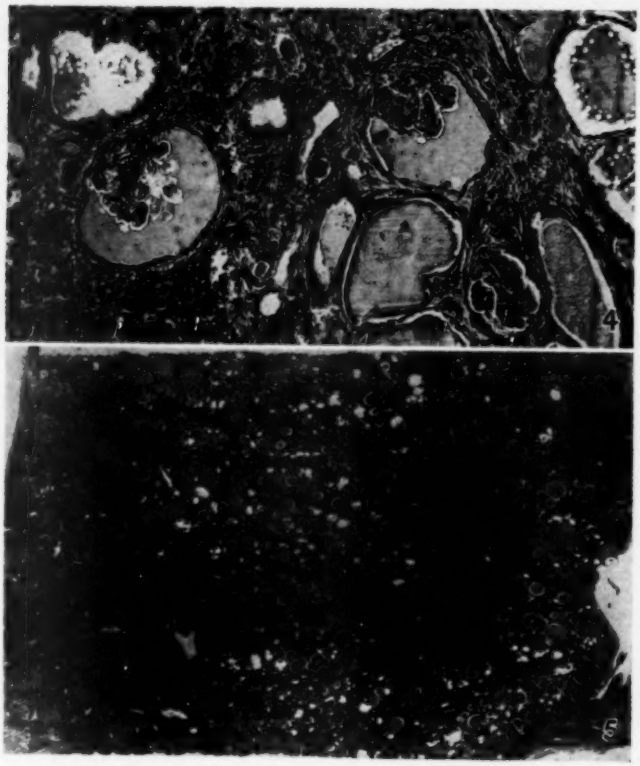


Fig. 4.—Cortex showing periglomerular fibrosis with shrunken tufts and "colloid" material in the glomerulus; × 100.

Fig. 5.—Cysts filled with "colloid" in cortex and medulla; approximately × 4.

Many of the cystic spaces, ranging from 50 microns to 1 mm. in diameter, could be followed in a linear fashion for some length in both cortex and medulla. They were separated only by thin septums or partial septums without the cortical localization of the pyelonephritic contracted kidney but rather the diffuse distribution seen in polycystic kidneys (fig. 5).

For these reasons I believe that this case should be regarded as one of a form of congenital cystic kidney.

The reconstructive works of McKenna and Kampmeier,[3] Norris and Herman[4] and Lambert[5] largely disprove that cystic kidneys develop because of the failure of the two anlages to unite, incomplete development of the tubules or fibrotic obstruction of tubules.

Kampmeier[6] and later McKenna and Kampmeier[3] demonstrated that the first three or four generations of uriniferous tubules are not permanent but break away from their respective collecting ducts and undergo cystic degeneration during normal development of the kidney. They suggested that persistence and growth of these fetal cystic structures offer an explanation of the origin of cystic kidneys.

According to Norris and Herman,[4] there is normal development of the kidneys for a long period of fetal life, and focal cystic dilatation of uriniferous tubules and collecting tubules occurs after differentiation and union of the anlages; thus there is offered an explanation for the presence of cysts arising in the collecting tubules as well as in the uriniferous elements.

The inflammatory changes which finally led to renal failure in this case are best regarded as secondary interstitial nephritis, a not uncommon complication of congenital cystic kidney.

SUMMARY

A case of microcystic, thyroid-like kidney is reported. The patient was a 22 year old Negro woman. The cut surface resembled diffuse colloid goiter, and cystic tubules were found in the cortex as well as in the medulla.

Both kidneys were of normal size and did not present the usual evidence of chronic pyelonephritis, which is generally regarded as the cause of thyroid-like kidneys. From the study of this case it is concluded that not all thyroid-like kidneys can be attributed to blockage of the collecting tubules following chronic pyelonephritis, but that some of these kidneys are best interpreted as a congenital malformation of renal tubules.

3. McKenna, C. M., and Kampmeier, O. F.: J. Urol. **32**:37, 1934.
4. Norris, R. F., and Herman, L.: J. Urol. **46**:147, 1941.
5. Lambert, P. P.: Arch. Path. **44**:34, 1947.
6. Kampmeier, O. F.: Surg., Gynec. & Obst. **36**:208, 1923.

RUPTURE OF THE CORONARY SINUS FOLLOWING MYOCARDIAL INFARCTION

DAVID B. HINSHAW, M.D.
AND
ALBERT F. BROWN, M.D.
LOS ANGELES

REFERENCES to the coronary sinus or great vein of the heart are scarce in medical literature. Various investigations have been carried out in regard to the physiologic function of this structure. It is generally stated that about 60 per cent of the venous blood return from the heart muscle is by way of the coronary sinus—the remaining venous return being by way of the thebesian vessels and through direct communications between the coronary arteries and the ventricular cavities. The coronary sinus is not subject to pathologic changes to any appreciable extent. External trauma would perhaps comprise the major source of its pathologic changes.

The case reported in the following pages is one in which a myocardial infarction due to coronary thrombosis resulted in a rupture of the coronary sinus. No references to any previous instances of rupture of the coronary sinus could be found in the *Index Catalogue of the Surgeon General's Office* and the *Quarterly Cumulative Index Medicus.*

REPORT OF A CASE

The records of this case have been used with the permission of Dr. B. P. Mundall.

V. G., a 72 year old white Caucasian man of medium height and weight, was admitted to the Glendale Sanitarium and Hospital in February 1943. He had had recurrent attacks of pyelonephritis for three years; also, personality changes had been noted by his family for several months. His chief complaint on being admitted was severe thoracic pain radiating to the left arm and the upper part of the back. Opiates were required to relieve this pain.

The pulse rate was 100 per minute, the respiratory rate 28 per minute and the blood pressure 140 mm. of mercury systolic and 90 mm. diastolic. Heart, lungs and abdomen were not unusual. No edema was present. The reflexes were all somewhat hyperactive but equal bilaterally; both Babinski reactions were equivocal. The prostate gland was firm and moderately enlarged; no rectal masses were felt.

The urine was cloudy and on microscopic examination contained moderate numbers of pus cells and red blood cells; no sugar or albumin was present. The red blood cell count was 4,800,000; the hemoglobin, 17 Gm.; the white blood cell count was 8,900, 89 per cent of which were mature neutrophils. The nonprotein nitrogen of the blood amounted to 124 mg. per hundred cubic centimeters.

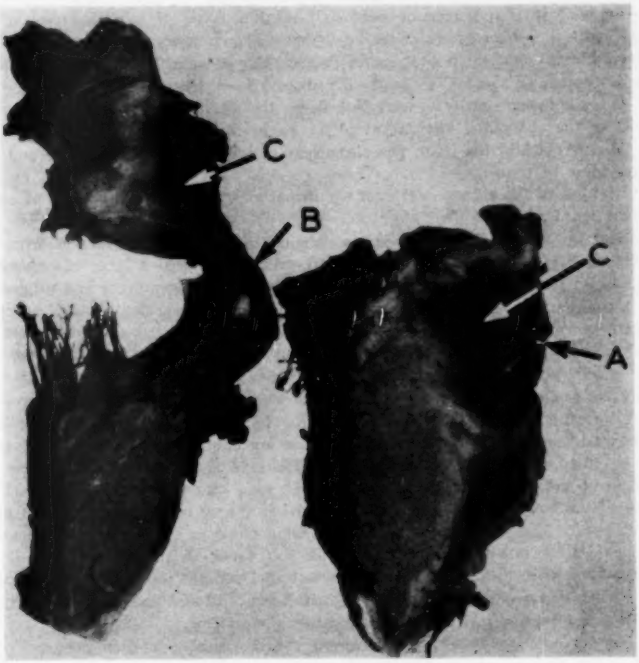
The patient became progressively worse. The thoracic pain continued, and his temperature varied between 99 and 101 F. He became irrational and died thirteeen days after admittance.

From the Departments of Pathology and Medicine, Glendale Sanitarium and Hospital and College of Medical Evangelists.

Autopsy.—The body was that of a well developed, moderately well nourished white man aged 72 years. No external findings of importance were to be noted.

Cross sections of the brain had areas of moderately old necrosis with partial cystic degeneration in the corpus striatum of each side. Further sections of the brain showed a 6 mm. hemorrhagic discoloration in the cortex of the right parietal occipital region, apparently due to recent infarction.

The pleural cavities were without significant change. The lungs were moderately heavy and on the cut surface showed edema and congestion. The pericardial sac



Cross sections of lateral and posterior portions of the left ventricle showing the coronary sinus distended with blood and communicating with an area of hemorrhagic infiltration of the epicardium. *A* indicates the area of rupture of the terminal portion of the coronary sinus; *B*, a cardiac aneurysm; *C*, the coronary sinus.

was distended with clotted blood. Some of this clot was soft and red, apparently fresh. Other portions of the clot showed discoloration and suggestive platelet lines, indicating older hemorrhage. This portion of the clot was strongly adherent to the epicardium.

The heart was approximately normal in size. The ventricular cavities were not dilated. The wall of the left ventricle was 1.2 cm. thick except in the upper lateral portion of the left ventricle where the wall was scarred, thinned to 5 mm. and pouched outward. No evidence of rupture was found in this area, but the scar tissue showed hemorrhage and discoloration, suggestive of recurrent necrosis.

There was an aneurysmal sac about 2 cm. in diameter and about 1 cm. deep. The remainder of the lateral wall of the left ventricle showed a large area of moderately recent infarction in which the myocardium was discolored yellow-gray and had a somewhat gelatinous texture. No point of rupture was found in this area. The epicardium was especially involved in the hemorrhagic process along the lateral left atrioventricular margin. The coronary sinus in this region appeared to be greatly distended with clotted blood, and there was a suggestion of a defect in its wall in an area continuous with the region of intraepicardial hemorrhage. The wall of the coronary sinus at this point was also in close relation to the old aneurysmal scar and area of necrosis. A thin layer of mural thrombus was attached to the inner aspect of the aneurysm. The coronary arteries were severely atherosclerotic, and the left circumflex branch was occluded by a pink-red thrombus for a distance of about 1 cm. beginning about 1.5 cm. from its origin.

No significant changes were found in the peritoneum, the gastrointestinal tract or the abdominal viscera except for slight chronic passive congestion.

Microscopically the brain revealed areas of moderately old softening and disintegration of tissue.

Sections of the kidneys were not significant except for a small amount of edema of the renal cortex.

Sections of the heart warrant a detailed description. A section from the midportion of the lateral wall of the left ventricle showed a layer of organizing blood clot covering the epicardial surface. The organizing process had progressed to a considerable depth in the clot. The underlying myocardium showed complete recent necrosis which involved all but narrow layers near the epicardium and the endocardium. Large areas of undissolved myocardial fibers were still present in the infarct, but there was much granulation tissue and early fibrosis, especially near the blood vessels. One area showed older fibrous tissue, apparently that of a previous scar. Sections taken from the base of the left ventricle through the coronary sinus and the upper border of the old cardiac aneurysm showed the coronary sinus to be filled with recent blood clot. Portions of the clot which lay near the periphery exhibited platelet lamination and some degree of organization. The wall of the coronary sinus appeared involved in the necrosis of the adjacent myocardium and had apparently ruptured, allowing blood to dissect into the epicardial tissues. A peculiar hyperplastic reaction was found in the mesothelium of the epicardium, with the formation of multinucleated cells in some places. A portion of the left circumflex coronary artery, which was included in one of the sections, showed severe atherosclerosis and thrombotic occlusion.

Anatomic Diagnoses.—Arteriosclerotic heart disease; coronary thrombosis; old myocardial infarction with cardiac aneurysm; recent myocardial infarction; rupture of the coronary sinus due to its having been included in the area of infarction; organizing hemopericardium; mural thrombus of the left ventricle; cerebral softening, possibly embolic.

SUMMARY

A case of rupture of the coronary sinus of the heart associated with myocardial infarction is presented. As nearly as can be determined, no similar case has as yet been described in medical literature.

Laboratory Methods and Technical Notes

RETICULUM SILVER IMPREGNATION FOR OLD FORMALDEHYDE-FIXED TISSUE

ELENA DE GALANTHA
HOUSTON, TEXAS

THE usual methods employed for the staining of reticulum are not applicable to tissue that has been kept in formaldehyde solution U.S.P. for long periods. The method to be described has given good results with tissue kept in formaldehyde solution as long as ten to twenty years.

METHOD

1. Cut in half-centimeter square blocks.
2. Rinse free of formaldehyde solution in tap water (two hours).
3. Put blocks in 3 per cent strong ammonia solution U.S.P. for twenty-four hours.
4. Wrap each block in a piece of gauze and put the wrapped blocks in a dish under slowly running tap water for twenty-four hours.
5. Dehydrate in 75 per cent alcohol for one hour.
6. Dehydrate in 95 per cent alcohol for one hour.
7. Place in acetone for three hours.
8. Clear in xylene for one hour.
9. Impregnate with soft paraffin for one hour.
10. Embed.
11. Cut sections at 5 to 6 microns.
12. Dry in a 37 C. oven overnight.
13. Deparaffinize in xylene (two changes) and 95 per cent alcohol, then pass through chloral hydrate (saturated solution) and into distilled water.
14. Place slides in coplin jars with 5 per cent silver nitrate for twenty-four hours in a 37 C. oven.
15. Wash quickly in distilled water.
16. Place slides in silver ammonium oxide for five to ten minutes. (Silver ammonium oxide is prepared as follows: to 100 cc. of 5 per cent silver nitrate add 5 drops of 40 per cent sodium hydroxide. Clear with strong ammonia solution U.S.P., drop by drop, and shake until clear.)
17. Wash slides in distilled water carefully and place them in a solution made of 1 part of 40 per cent formaldehyde solution to 9 parts of tap water, for ten minutes.
18. Wash quickly in distilled water.

From the Department of Pathology, M. D. Anderson Hospital, University of Texas.

19. Carefully reduce the excess of precipitate in 1 per cent gold chloride (brown) with 2 drops of acetic acid.

20. Wash in water quickly, clear in 5 per cent sodium hyposulphite for one minute and rinse quickly in distilled water.

21. Clear in 50, 70, 80 and 95 per cent alcohol and xylene. Apply cover slip with Canada balsam.

NOTE: This method is good for fresh formaldehyde-fixed tissue, but it does not need the prolonged process. Avoid nos. 2, 3 and 4. In no. 14 reduce the time to five hours.

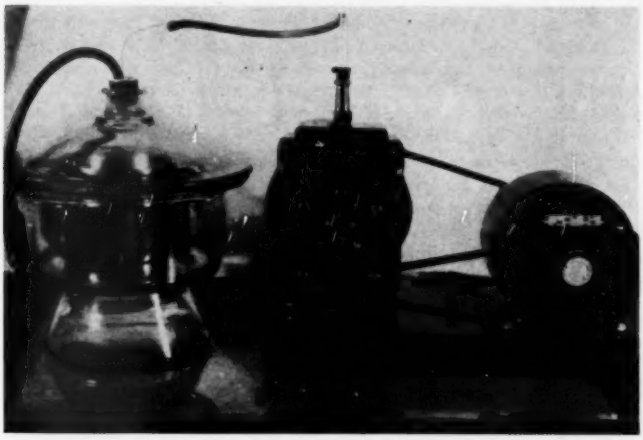
RESULTS

Reticulum is stained intense black, and the background is almost colorless.

TECHNIC OF VACUUM PARAFFIN INFILTRATION OF TISSUE ADAPTED TO THE USE OF THE TECHNICON

CRICHTON McNEIL, M.D.
SALT LAKE CITY

AFTER fixation and dehydration, tissues must be exposed to paraffin for two to five hours, depending on the size of the section, to obtain satisfactory embedding. Pathologic laboratories have made use of vacuum to obtain better paraffin infiltration,[1] but no reports are available and no attempt to adapt this technic to the use of the automatic "technicon" is known.



The vacuum type desiccator jar at the left holds the "technicon" paraffin container and basket. Electrical connection is maintained through a rubber stopper, which also carries the exhaust tube directly to the vacuum pump.

PROCEDURE

Tissues are placed in the "technicon" basket at jar 1, which contains a 4 per cent formaldehyde solution in 70 per cent ethyl alcohol, where they remain until 9 p.m. Thereafter until 7 a.m. they are passed at hourly intervals through the usual graded alcohols, dioxane, dioxane and paraffin, and finally into paraffin. At 8 a.m. the entire heated paraffin jar with the tissue basket is detached from the rotator and placed in the 200 mm. diameter Scheibler desiccator jar, which has a two hole rubber stopper in the lid (figure). One outlet is attached to the vacuum

From the Laboratories of Holy Cross Hospital and the Department of Pathology, University of Utah School of Medicine.

1. Landau, E.: Bull. d'histol. appliq. a la physiol. **16**:13, 1939.

pump [2]; the other, containing the wire, is plugged into a regular electrical outlet. Vacuum is quickly obtained and the motor turned off so that too great a vacuum and foaming are avoided. This negative pressure corresponds to a mercury column of 294 mm.

Experience has shown that in this vacuum a one-half hour exposure of tissues is adequate for complete impregnation. The tissues are blocked as usual.

The following advantages have been observed: Tissues cut with greater ease, and flaws are eliminated; thinner sections can be obtained, because of better impregnation; in the "technicon" there is more time for dehydration; tissues are not "cooked" in paraffin.

2. The W. M. Welch Manufacturing Company Duo Vacuum pump model 1400 B is used.

Books Received

ZINSSER'S TEXTBOOK OF BACTERIOLOGY: THE APPLICATION OF BACTERIOLOGY AND IMMUNOLOGY TO THE DIAGNOSIS, SPECIFIC THERAPY AND PREVENTION OF INFECTIOUS DISEASES FOR STUDENTS AND PRACTITIONERS OF MEDICINE AND PUBLIC HEALTH. Revised by David T. Smith, M.D., professor of bacteriology and associate professor of medicine, Duke University School of Medicine; Donald S. Martin, M.D., M.P.H., professor of preventive medicine and public health and associate professor of bacteriology, Duke University School of Medicine; Norman F. Conant, Ph.D., professor of mycology and associate professor of bacteriology, Duke University School of Medicine; Joseph W. Beard, M.D., professor of surgery in charge of experimental surgery, Duke University School of Medicine; Grant Taylor, M.D., associate professor of bacteriology and associate professor of pediatrics, Duke University School of Medicine; Henry I. Kohn, Ph.D., M.D., surgeon, U. S. Public Health Service, assistant professor of physiology and pharmacology (on leave), Duke University School of Medicine; Mary A. Poston, M.A., instructor in bacteriology, Duke University School of Medicine. Ninth edition. Pp. 992, with 251 illustrations. Price $10. New York: Appleton-Century-Crofts, 1948.

The inheritance of the responsibility of authorship of an established textbook is a mixed blessing to the new authors. Patterns good and bad have been set, and precedent discourages their alteration. Changes must be gradual, if due respect is to be shown for the judgment of past authors. This consideration exercises more influence in some instances than in others. So it is with considerable trepidation that this reviewer approaches the task of reviewing the current edition of the "Textbook of Bacteriology," first edited by Hiss, added to by Zinsser, then by Bayne-Jones, and currently by Smith and Martin.

In general the new edition retains the arrangement of the previous edition. New material has been introduced dealing with antibiotics and with pleuropneumonia-like organisms. In connection with the discussion of specific infections, new emphasis is placed on the public health significance of diseases. Revisions in text material have been made to include results of current investigations. By and large the material presented throughout the book is accurate, readable from a medical student's point of view, and valuable to any one interested in the subject of infectious diseases of man. Like most medical textbooks, it is too long. Failure to give differential emphasis to first things, and to save words by placing minutiae in appropriate tables, or eliminating them altogether, leads to criticism, although it certainly follows the precedent of current writing of medical textbooks. Many lines are wasted in describing isolated recoveries of pathogens from various animals, biologic properties which might be of interest at some future time, or morphologic or cultural variations not correlated with clinical problems. This is the type of information which the reviewer would prefer to see in tabular form, subject to reference but not impeding the reading of the student who seeks knowledge of the principles of medical bacteriology. The physical sciences have long since given up describing details in the text and instead relegate such information to appropriate tables or charts. A textbook must be read by students. One wonders, therefore, why the authors incorporate six pages of detail on the antigenic configuration of the salmonella in the body of the book. Could this material not better be placed in an appendix, so as not to distract the student from the important job at hand?

The selection of references is certainly a difficult problem. Obviously it is not possible to cite all the books relating to a given topic; nevertheless, it is important that careful considration be given to avoiding provincialism. There are evidences of this error in the new edition, even as in practically any textbook. Certain English writers seem to have a happier faculty for selecting more universal reference lists. Since textbooks are used—or at least it is the hope of the publisher that they will be—in schools the country over, this criticism assumes a practical nature. Since a topic can be only incompletely documented with references, in the reviewer's opinion authors of textbooks should be careful to make sure that there is a fair geographic distribution of emphasis, provided this is indicated by the merit of the works.

The typography of the new edition is excellent, and monotony is avoided through the frequent introduction of significant charts and pictures. Many new illustrations have been added. Unfortunately, not all the pictures and charts are well reproduced, and a few appear to have been selected more for their uniqueness than for their value in illustrating a principle. The selecting of pictures for a textbook demands as much discrimination as that of the items to be included in the text. Dramatic or unusual pictures may interest the novice or the expert but they confuse the student. There is much good taste shown by the authors of this textbook in following the principle indicated. This holds particularly for the section dealing with the higher bacterial forms. On the other hand, some yielding to temptation is evident in the use of unusual pictures from the field of virology which may not seem worth while to all readers.

The importance of presenting general principles of medical bacteriology and immunology is acknowledged in varying degrees by different writers of textbooks. In general there is less tendency among bacteriologists than among pathologists to develop the general aspects of their topic before going into details of diseases. Since the principles are of prime importance, it is regretted that the authors have not given more consideration to the fundamentals of infection, resistance, immunology and the general physiology of micro-organisms. The pattern of the earlier editions possibly influenced the authors in their emphasis, yet another approach might have served equally well the purpose of developing a knowledge of principles on the part of users of the book. The problem of the degrees to which bacteria are destroyed by chemicals and antibiotics *in vitro* and *in vivo* is inadequately presented. Bacteriostatic action and bactericidal action of disinfectants are not well differentiated. No reference is made to some of the more recent experiments in determining the effectiveness of disinfectants by methods designed to differentiate between bactericidal and bacteriostatic action.

There are advantages and disadvantages in adding a section on practical methods to a textbook on bacteriology. If such a section is added, then it should be fairly complete. The reviewer believes that this section of the textbook in question is not as carefully prepared as it might have been, revealing a fair number of sins of omission and commission.

The reviewer, having exhausted the details of all that is or could be wrong with this book, now would like to indicate in general terms that this ninth edition of Zinsser's text, edited by Smith and Martin, can easily be considered a first class work among the available American textbooks in medical bacteriology. It is the reviewer's opinion that a great many of his criticisms could equally be leveled against many other textbooks of bacteriology in the field today. Certainly no medical student who is assigned this book as a text will fail to learn his subject through any failure of the book studied. Also, any medical graduate may find a great deal of valuable information in this book, readily available and presented in a readable manner. The evidence of the improvement of this text over the eighth edition is obvious.

Paragon Tray Drawer Cabinet

Compact

U. S. Pat. No. 2,202,047
C101—Tray Drawer Cabinet for 3 x 1 Micro Slides
Capacity 4500—18¾ x 15¾ x 4¼

Low Cost

FOR FILING
MICROSCOPIC SLIDES 3 x 1"
KODACHROME TRANSPARENCIES
2 x 2" SLIDES
LANTERN SLIDES (up to 3¼ x 4¼)
PETROGRAPHIC SLIDES

When you purchase a
PARAGON TRAY DRAWER CABINET YOU PURCHASE FILING SPACE ONLY NO WASTE SPACE-EVERY INCH USED

All Paragon Tray Drawer Cabinets are manufactured in standard sizes so that any number of sections may be interlocked to form one cabinet to accommodate any number of varied slides. The dimensions of the different cabinets are the same as to length and width, varying only in height. The cabinet formed by interlocking may be 18¾ x 15¾; 18¾ x 11 or 18¾ x 5 or it may be a pyramid with the sections varying in width.

C221—Capacity 1500 Slides—18¾ x 11 x 3⅜
For Filing KODACHROME TRANSPARENCIES and 2 x 2" SLIDES

SPECIFICATIONS: All Paragon Tray Drawer Cabinets are made of reinforced steel construction, olive green finish. Interlocking device enables several units to be joined into one. Each sectional unit contains removable drawers with hand grip in front and rear. Interlocking steel base obtainable whenever required. **Constructed according to rigid specifications—not merely adapted.**

Address your orders and inquiries to Dept. P.
Manufactured Exclusively by

PARAGON C. & C. CO., Inc. · 2540 Belmont Ave., New York 58, N. Y.